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The Journal of Laboratory and Clinical Medicine

VOL. XVII ST. LOUIS, MO., JULY, 1932 NO. 10

SYMPOSIUM ON HEMATOLOGY

(Concluded from June issue)

STUDIES ON THE STRUCTURE AND FUNCTION OF BONE MARROW*

I. VARIABILITY OF THE HEMOPOIETIC PATTERN AND CONSIDERATION OF METHOD FOR EXAMINATION

R. P. CUSTER, M.D., PHILADELPHIA, PA.

THERE is perhaps less consistency in the study of the bone marrow than of any other tissue of the body removed at autopsy for the study of diseases of the hemolytopoietic system. This is especially unfortunate and probably results from the difficulties encountered in removal and preparation. Not only is the tissue scattered widespread throughout the body and encased in a bony envelope that is difficult to remove, often necessitating an extra body incision, but, as will be shown later, its cellular state varies (more than any other tissue) in different localities, both in different bones and at different levels of the same bone; even a single cross-section at a given level shows wide inconsistency. Gross examination is often fallacious, in that congestion or hemorrhage may be indistinguishable from hemopoietic cellular hyperplasia. There is thus a considerable need for a standardized method of bone marrow study.

That the marrow has not been studied either sufficiently or efficiently during the course of routine autopsy work is shown by an analysis of the postmortem services of two teaching hospitals in this city. During the years 1927, 1928, and 1929, 2618 autopsies were performed. In this series, the bone marrow was examined 47 times, or in 1.7 per cent of cases. In 25 of these, the marrow of but one bone was observed; several instances simply grossly. For the most part the histologic descriptions merely confirmed the gross diagnosis of hyperplasia or normality, no mention of cytology being made. Examination of the slides showed that in many cases cytologic detail was extremely poor.

*From the Department of Pathology of the School of Medicine University of Pennsylvania and The Laboratories of the Philadelphia General Hospital, Philadelphia.

It is a well-known fact that during early childhood the marrow of practically all of the bones is of the cellular type and that this condition persists in the vertebrae, ribs, sternum, bones of the skull, and os innominatum, and to some extent in the proximal epiphyses of the femur and humerus, throughout life

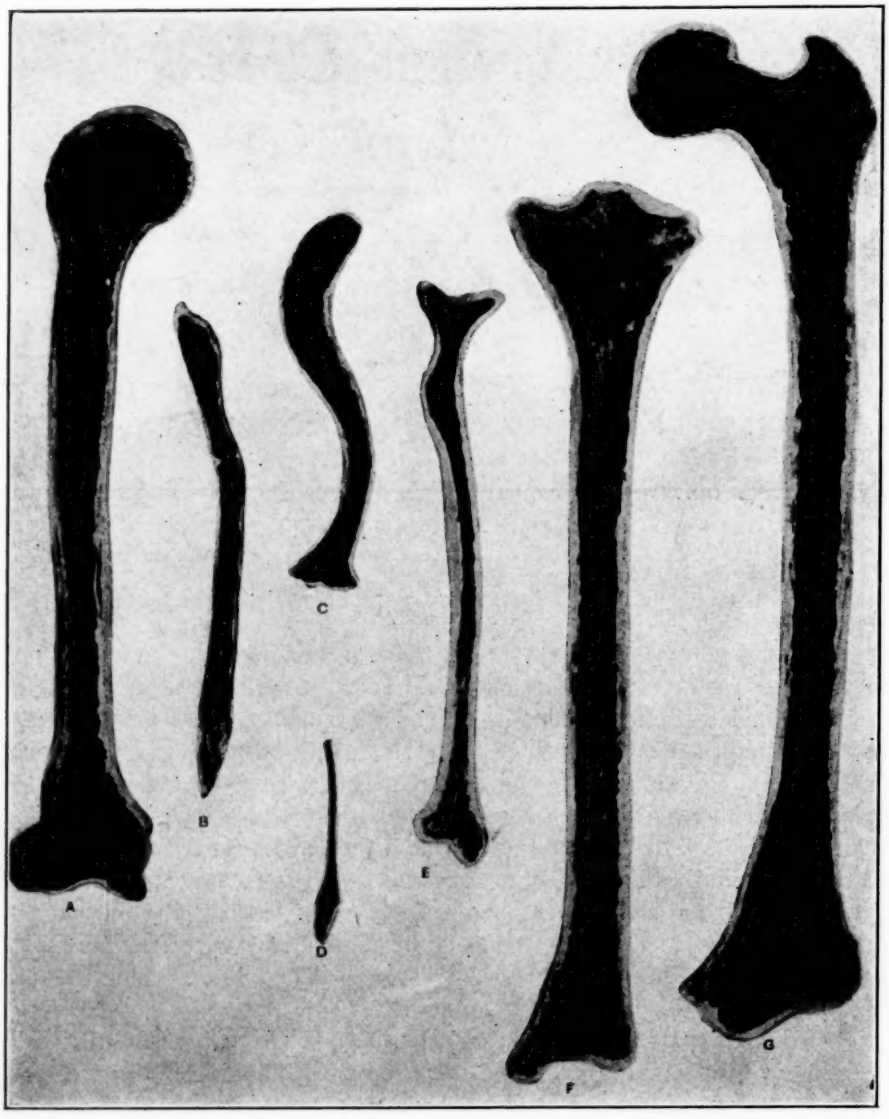

Fig. 1.—Characteristic bone marrow in pernicious anemia. *A*, left humerus; *B*, sternum; *C*, right clavicle; *D*, right fifth rib; *E*, right radius; *F*, left tibia; *G*, right femur. (The red of the hyperplastic marrow is here reproduced in black.) (Photographic reproduction of a color plate appearing in "Pernicious Anaemia and Aplastic Anaemia" by Arthur Sheard, through courtesy of William Wood and Company, American publishers.)

(Neumann[1]). Under normal conditions the shafts of the long bones assume the predominantly fatty and nearly acellular type of marrow beginning at the fifth to seventh year and becoming complete about the eighteenth year. This fat tissue, however, is in an extremely labile state and is capable of quite rapid replace-

ment by hemopoietic elements, beginning usually at the margin of the myeloid cavity and extending to the center as the need arises. I have observed in pigeons under experimental conditions the metamorphosis from an almost completely fatty marrow to a totally cellular marrow in the course of two days. I have seen

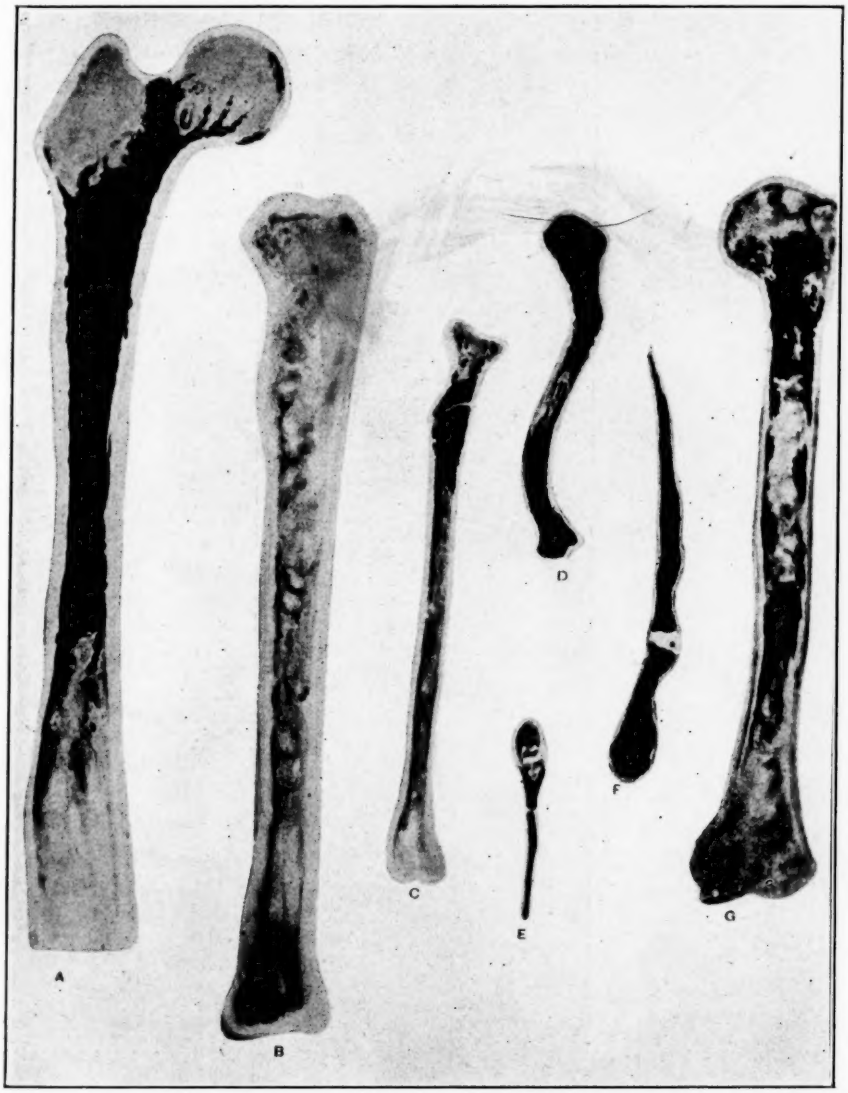

Fig. 2.—Bone marrow in pernicious anemia. Showing marked variation in hyperplasia, not only in different bones but at different levels of the same bone. The tibia is completely aplastic. *A*, right femur; *B*, left tibia; *C*, right radius; *D*, right clavicle; *E*, right fifth rib; *F*, sternum; *G*, left humerus. (Photographic reproduction of a color plate appearing in "Pernicious Anaemia and Aplastic Anaemia" by Arthur Sheard, through courtesy of William Wood and Company, American publishers.)

almost as rapid hyperplasia in dogs made anemic by injection of pyrodin. This hyperplasia of hemopoietic tissue does not occur with any degree of regularity, as mentioned previously. Two cases of pernicious anemia described by Sheard,[2] in which extensive examination of the marrow was made, are particularly illustra-

tive of this fact. Fig. 1 pictures the more usual findings in an untreated case, sometimes even a more solidly cellular marrow being found in the tibia and radius than is shown here. In contrast, Fig. 2 presents the marrow from a parallel case with equal need for blood cells in which the entire tibia and many levels

Fig. 3.—Bone marrow in secondary anemia. Showing wide variation in hyperplasia of the femoral marrow at different levels and parts of the cross-section. (Photographic reproduction of a color drawing.)

of the other bones are nearly acellular. Had the tibia or the distal end of the femoral shaft alone been examined in this case, an erroneous conclusion would have been reached, perhaps throwing the case into the category of the aplastic anemias. Surprisingly, in the latter case, the proximal epiphyses of the humerus and femur, normally cellular, are seen grossly to be aplastic, this finding having

been confirmed histologically. Fig. 3 (author's case) shows the typical irregularity of distribution of blood-forming tissue in secondary anemia of moderate grade. Fig. 4, a low-power photograph of a longitudinal section of femoral marrow from a case of carcinomatosis with cachexia, pictures an abrupt line of demarcation between an 80 per cent hyperplastic marrow and a gelatinous degeneration.

Strumia,[3] in a report of several cases of myelogenous leucemia, has made pertinent observations on this question. In one of his cases "the bone marrow of both tibiae, upper and lower portions, was yellow, fatty, and contained spongy bone. It appeared altogether as nonfunctioning adult bone marrow. The marrow of the bones of both feet was identical in structure and appearance with the bone marrow of the tibia. The bone marrow of the femur and of the sternum was red and gelatinous with few hemorrhagic points." Carefully prepared sections and smears of each of these bones showed those appearing grossly fatty to have few cells grouped in small foci, while the femur and sternum were hyper-

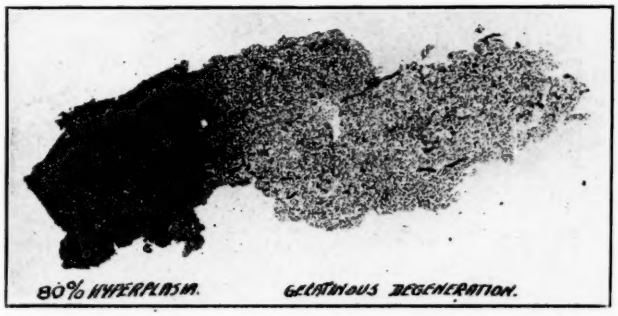

Fig. 4.—Longitudinal section of femoral marrow. (×4.5.) From a case of carcinomatosis with cachexia, showing an abrupt line of demarcation between an 80 per cent hyperplasia and gelatinous degeneration.

plastic. Differential counts showed approximately the same percentages in each bone. Strumia states further, "Aplastic bone marrow in the tibia accompanied by hyperplastic changes in other bones has been observed by the author in a long series of cases including all forms of anemia, acute and chronic leucemia, and acute and chronic inflammatory processes, especially sepsis, accompanied by profound changes of the blood picture. It appears, therefore, that the bone marrow of the tibia is a poor index of the general conditions of the bone marrow. Since the bone marrow of the tibia is readily accessible, it has been often depended upon at autopsies to indicate the general condition of the bone marrow; a practice which should be discontinued."

Confirmative of this latter observation by Strumia are photomicrographs taken from one of our recent cases (Figs. 5 to 9 inclusive), a case of bacterial endocarditis with marked secondary anemia. Particularly striking is the difference in cellularity between two levels of the femur 1 centimeter distant from each other. Hemopoietic tissue in the tibia is nonexistent. The sternum and rib each show an increased cellularity over their normal adult state.

The inconsistency in the tissue itself should be sufficient indication for a careful and uniform technic and a survey of more than one bone. On the con-

trary, however, except when performed by those workers carrying out special studies on the bone marrow, the reverse is too often the case. For example, the tissue selected for study by many pathologists is removed at random from any one convenient bone, perhaps the tibia on account of its availability. Often one notes in protocols that the bone used is not even mentioned. Fixation in 10 per cent unneutralized formol is usual. The presence of bone spicules makes it necessary to subject the tissue to destructive decalcification. The marrow is then embedded in paraffin, cut at too great a thickness, and stained with hematoxylin and eosin. Consequently, the sections are unsatisfactory for study and often meaningless. Disappointing results naturally lead to a neglect of the tissue.

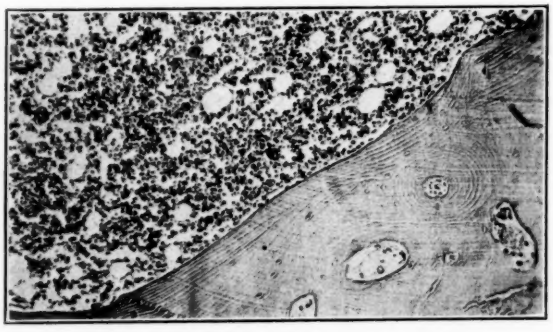

Fig. 5.

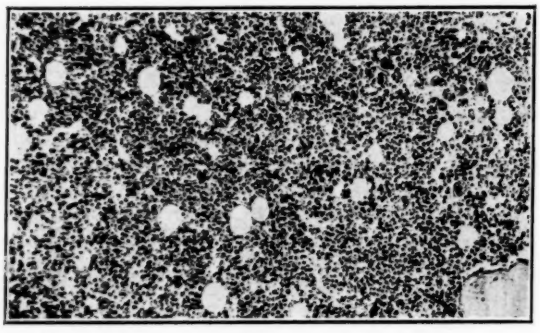

Fig. 6.

Figs. 5 and 6.—Bone marrow from case of bacterial endocarditis, showing almost complete cellularity. Fig. 5.—Rib marrow. Fig. 6.—Sternal marrow.

METHODS

The technic that has been found most satisfactory and that is productive of most uniform results is outlined in rather brief form as follows:

Removal of Tissue.—If but one specimen of marrow is to be taken, the mid-femur is the bone of choice. Expose the midportion of the shaft through a horseshoe incision, through skin, fascia, and muscle, make parallel saw-cuts halfway through the bone about three inches apart, and chisel off the cortex between the two cuts (being careful not to break the bone completely through). The marrow cavity is thus exposed and the pencil of marrow may be lifted out intact with a gouge or similarly curved instrument. This will give sufficient tissue for

both cross and longitudinal sections through the marrow. The portion selected for fixation and embedding should be free of bone spicules if possible. Marrow from the tibia may be removed in a similar manner from the middle of the shaft, although the piece need not be so large. The sternum specimen should be taken through the gladiolus between transverse cuts not over five millimeters apart. Care must be exercised in taking rib marrow, as undue pressure on the bone will squeeze the tissue from the myeloid cavity. It is best to cut a piece of the rib

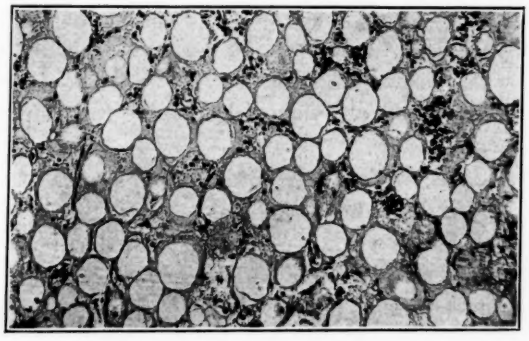

Fig. 7.—Tibial marrow. The clumps of cells are adult erythrocytes within dilated blood channels.

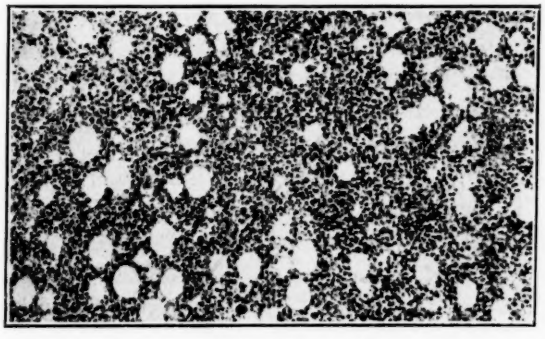

Fig. 8.

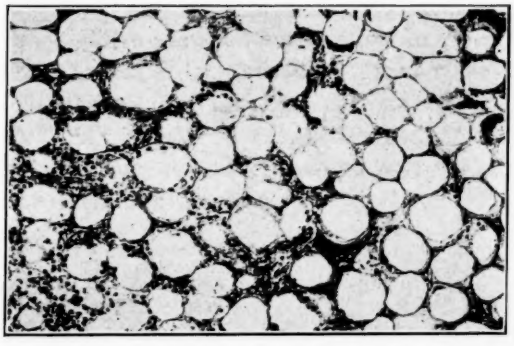

Fig. 9.

Figs. 8 and 9.—Two levels of femoral marrow. One centimeter distant from each other, showing wide variation in cellularity.

five to seven millimeters in length with a fine hack-saw and section the bone intact. A thin wedge of a lumbar vertebral body may be removed without difficulty.

Fixation.—This step is of great importance and it is imperative to have the tissue removed as soon after death as possible (within eighteen hours the fine cell structures may be lost and the best results can be expected only if the marrow is obtained within eight hours). The fixation of any hemopoietic tissue is most perfect in Zenker-formol solution, composed of nine parts of freshly prepared Zenker's solution (without acetic acid) to which one part of neutral formol (40 per cent formaldehyde over magnesium carbonate) is added within one-half hour after the tissue has been placed in the Zenker's solution. Fixation should extend over four to twelve hours, followed by twelve to twenty-four hours washing in running water.*

Decalcification.—Marrow from the sternum, rib, vertebra, and occasionally from the tibia must be lightly decalcified. For good preservation of cell structure this is best carried out in a mixture of equal parts of an 85 per cent aqueous solution of formic acid and a 20 per cent aqueous solution of sodium citrate. Twelve to twenty-four hours is usually sufficient time to decalcify cancellous bone, the cortex of which has been trimmed away. Decalcification with 5 per cent potassium bichromate gives excellent results but the process often requires several weeks. If this method is used, the solution should not be changed more than once. The tissue must be washed well in running water after decalcification.

Dehydration.—The tissue is carried through graded alcohols, beginning with 65 per cent and passing through 80, 90, 95 per cent, absolute (two changes) and equal parts of absolute alcohol and ether. The first two contain iodine to dissolve the mercuric bichloride of the fixative and the last two must stand in a desiccator over anhydrous copper sulphate to insure perfect dehydration.

Embedding.—Celloidin is by all means the best, as most of the cell shrinkage is avoided. The method described by Addison in "Piersol's Normal Histology"[4] is most satisfactory. Paraffin may be used and quite good sections obtained, although cytoplasmic detail is not so well preserved as with celloidin. To cut thin sections with celloidin, an anhydrous technic must be strictly observed in the preparation of the materials; otherwise, the celloidin will be rubbery and impossible to section at less than eight microns thickness.†

Staining.—A variety of staining methods are available, of which the azurII-eosin is most consistently good.** Structural detail of nucleus and cytoplasm brought out clearly by this stain is quite lost in preparations stained with hematoxylin-eosin. Details of the method may be found in McClung's "Microscopical Technique."[5] A modification of the Ellermann method described by Richter[6] gives beautiful results, but it is more difficult and less consistent. The eosin-

*The stains recommended are not applicable to formol-fixed tissue. This may be remedied in part by soaking the cut sections in acetic-free Zenker's solution for about twelve hours; the sections should be washed in running water for several hours and transferred through two weak solutions of iodine in alcohol; finally, through several changes of 95 per cent alcohol to remove the iodine.

†Sectioning of the tissue should be done at a thickness of 4 microns in paraffin and 5 or 6 in celloidin. The sections must be mounted on albuminized slides from the knife and the celloidin removed before staining. Transfer the mounted section through the following solutions: 95 per cent alcohol with iodine (2 changes); 95 per cent alcohol (2 changes); absolute alcohol; absolute alcohol and ether (equal parts); 95 per cent alcohol; 80 per cent alcohol; 60 per cent alcohol; 30 per cent alcohol; water (3 changes); stain.

**AzurII as prepared by the National Aniline and Chemical Company is best.

methylene blue stain is quite good. Sections stained with Wright's blood stain or Pappenheim's modification of the May-Grünewald-Giemsa stain are quite satisfactory.

Mounting.—Richter has stated that euparol-mounted preparations are more permanent than those mounted in balsam, the azur having less tendency to fade. We have found neutral balsam quite satisfactory, however, and for ordinary work recommend its use. Gum damar is also good.

Supravital Preparations.—The cells of the bone marrow survive for six or seven hours after death and can be studied by the supravital staining technic during this time. A piece of the tissue of about the size of two pinheads is cut with fine curved scissors and carefully transferred to the cover-slip which has been prepared with vital stain according to the technic of Sabin.[7] The tissue must not be handled with forceps. Apply the slip to a slide with very gentle pressure until the tissue is flattened out. Examination of the main piece allows the tissue between the fat cells to be analyzed, while the free cells along the edges permit of a differential count.

Teased Preparations.—Motility of the marrow cells can be observed in teased preparations made soon after death. A tiny bit of marrow from the rib, the size of a pinhead, is squeezed out into a drop of blood serum on a cover-slip and mixed gently. The slip is inverted into the chamber of a hanging-drop slide and the slide transferred promptly to a warm-stage microscope. Cell types are difficult to identify in these preparations. Larger amounts of the marrow may be teased out of the rib, mixed with blood serum menstruum, and smears made in a fashion similar to blood smear preparations. This should be fixed before drying and stained either as a blood smear or as a tissue section.

SUMMARY AND CONCLUSIONS

1. The bone marrow as a tissue presents unusual difficulties for study, particularly due to its delicacy, complexity, inaccessibility, and wide distribution. Its cellular state varies widely in different bones, different levels of the same bone and different areas of a cross-section through a given level.

2. The bone marrow in diseases of the hemolytopoietic system has hitherto been more often haphazardly studied than otherwise.

3. A method is presented, the use of which will result in greater accuracy and consistency in study of the bone marrow.

4. It is hoped that study of the bone marrow by pathologists conducting a routine autopsy service will make available more data relating to diseases of the hemolytopoietic system.

I am indebted to Mr. B. B. Varian of the Department of Anatomy for assistance in developing adequate microscopic technic.

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STUDIES ON THE STRUCTURE AND FUNCTION OF BONE MARROW

II. Variations in Cellularity in Various Bones With Advancing Years of Life and Their Relative Response to Stimuli*

R. P. Custer, M.D., and Florence E. Ahlfeldt, M.D., Philadelphia, Pa.

IN A previous paper, attention was called to the fact that study of the bone marrow should not be confined to a sample removed from a single bone. The tibia, femur, rib, sternum and vertebra, as a group, were suggested as preferable bones for marrow study. In the event that such an extensive survey was not considered necessary in an individual case, the femur and vertebra were recommended as the choice bones for examination, the former to determine the response of the blood-forming organs to stimulation, by metamorphosis from fatty to red marrow, the latter to study the cell content.

To establish a rough base line for expectant cellularity at a given age and to determine the relative response of the marrow of the various bones in the face of stimulation, the tibia, femur, rib, sternum and vertebra were observed in a series of one hundred cases, the results being shown graphically on the appended chart. Obviously this can represent only an approximate result; under no circumstances can it be applied with mathematical accuracy to a given case. The number of cases studied in each decade of life are noted in Table I.

Table I

Age Distribution Based on 83 of the Cases

Under 1 month	7
1 month to 1 year	11
1 year to 10 years	9
10 years to 20 years	4
20 years to 30 years	5
30 years to 40 years	9
40 years to 50 years	6
50 years to 60 years	13
60 years to 70 years	12
70 years to 80 years	6
80 years to 90 years	1

CONCLUSIONS

1. Study of the tibia, femur, rib, sternum and vertebra in 100 unselected cases shows that the cellularity of these marrows decreases with advancing years of life, the decrease corresponding in rapidity to the order named. The response

*From the Department of Pathology, University of Pennsylvania School of Medicine and the Division of Pathology of the Philadelphia General Hospital.

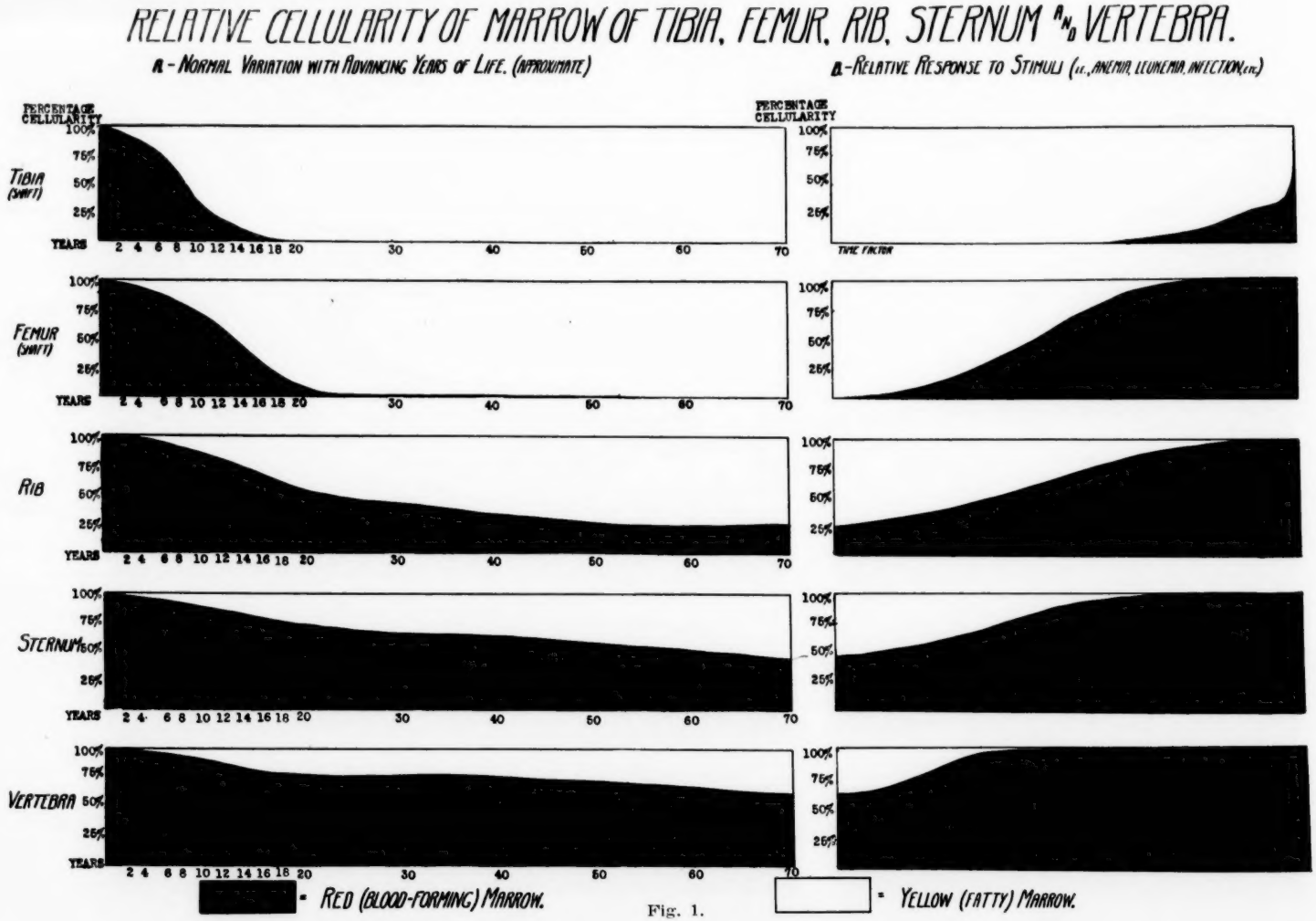
RELATIVE CELLULARITY OF MARROW OF TIBIA, FEMUR, RIB, STERNUM AND VERTEBRA.
A - NORMAL VARIATION WITH ADVANCING YEARS OF LIFE. (APPROXIMATE)
B - RELATIVE RESPONSE TO STIMULI (i.e., ANEMIA, LEUKEMIA, INFECTION, etc.)
PERCENTAGE CELLULARITY
100%
75%
50%
25%
TIBIA (SHAFT)
FEMUR (SHAFT)
RIB
STERNUM
VERTEBRA
YEARS
2 4 6 8 10 12 14 16 18 20 30 40 50 60 70
TIME FACTOR
= RED (BLOOD-FORMING) MARROW.
= YELLOW (FATTY) MARROW.

Fig. 1.

of these marrows to a hemopoietic stimulus of a given intensity is in inverse order, except in the case of the femur, which appears more labile than the rib.

2. Bone marrow taken for histologic study at biopsy or necropsy should be selected with these points in mind.

3. The previously expressed opinion that the mid-femur is the best single marrow and that femur and vertebra are the best combination for study is confirmed. The sternum is the most suitable bone for biopsy.

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Custer, R. P.: J. Lab. & Clin. Med. 17: 951, 1932.

A NEUTROPHILIC GRAPH*

Robert J. Needles, M.D., Detroit, Mich.

OUR present knowledge of the blood picture in health and disease is, at best, none too complete; it is generously spotted with areas of conjecture. Because of this, any article attempting to deal with the entire field of infection and its effect on the blood would, of necessity, be in the nature of a forecast and not a statement of facts. It is felt, however, that clinicians have not realized the importance of a complete neutrophilic differentiation by one method or another. It is the purpose of this paper to add to the literature a new series of complete neutrophilic differential counts; to give further indication of how such a nuclear partition may be of help in diagnosis and prognosis; and to present a new method of graphic representation of the percentage of neutrophiles of different age groups as they appear in the differential count.

Since Arneth, in 1904,[1] first demonstrated the clinical significance of the different nuclear forms of the polymorphonuclear neutrophilic leucocytes, the subject has received sporadic attention. Schilling, in 1920,[2] following the general plan laid down by Arneth, but establishing different criteria for the estimation of hematopoietic activity, evolved a plan which has attained not a little recognition. In 1924 Pons and Krumbhaar[3] contributed still another plan of nuclear enumeration, in an attempt to simplify the former methods. Cooke and Ponder, in 1927,[4] made definite contributions, and Cooke laid down certain criteria for the definite placing of the polymorphonuclear neutrophiles in the proper age groups. Finally, in 1930, Farley et al.[5] presented a new series of normals and contributed another series of pathologic cases, attempting to simplify the methods of previous workers in the field.

TECHNIC

Blood smears are prepared in the ordinary manner, using finger tip or ear blood. It seems immaterial whether slide preparations or cover-slip preparations are used. Either is subject to a certain percentage of error, and neither is of much value unless properly done. The chief essentials are a thin, even smear,

*From the Department of Medicine and the Department of Laboratories, Henry Ford Hospital.

avoiding margination as far as possible. Wright's stain gives quite good results, but for the best differentiation of nuclei, Giemsa's method is preferred. In counting, 100 consecutive white blood cells are classified, as in an ordinary differential, except that as the neutrophiles are noted, they are placed in the particular age class to which their nuclear lobulation makes them eligible. The time required is but little longer than for the old type of differential count, since it is only necessary to determine the number of true lobules in the polymorphonuclear neutrophilic nucleus to definitely place it. As to the number of cells to be counted, I have given different numbers a rather thorough trial, as well as to check various counts for accuracy, and it seems fair to state that with a good smear, a careful count of 100 cells is sufficient.

Cooke's criterion reads "If there is any band of nuclear material except a fine filament connecting the different parts of a nucleus, that nucleus, for the purposes of the count, cannot be said to be divided." Hence, the neutrophiles naturally fall into the five classes as illustrated. Normals for this group have

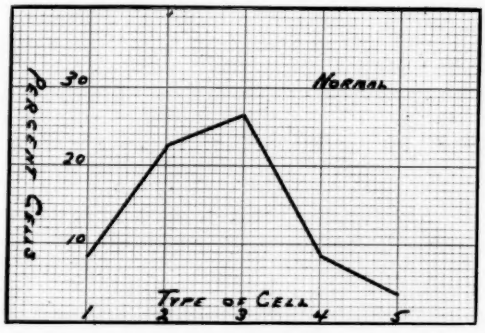

Fig. 1.—Total white blood count 7200, polymorphonuclears 70, small lymphocytes 22, large lymphocytes 3, monocytes 3, eosinophiles 1.5, basophiles 0.5. This figure is to illustrate the normal neutrophilic partition. Only neutrophiles are plotted, showing Class 1, 8.5; Class 2, 22.5; Class 3, 26.7; Class 4, 8.5; and Class 5, 3.5.

been stated by Cooke and Ponder to be 6.08 per cent of the first, or nonfilament group, while Farley et al. found 9.2 per cent. In 100 normal cases, with total counts ranging from 6000 to 8000, my normals average as follows:

POLYMORPHONUCLEARS 70.05									
1	2	3	4	5	SMALL LYMPHOCYTES	LARGE LYMPHOCYTES	MONOCYTES	EOSINOPHILES	BASOPHILES
8.45	22.99	26.7	8.7	3.21	20.32	4.32	3.24	1.57	0.5

The above figures are average. It should be understood that there is a normal range varying from 5 to 7 cells per hundred above and below each enumeration. All counts used in compiling these figures were from members of the nursing and service staffs of the hospital. In order to obtain one hundred normal counts it was necessary to examine about one hundred and thirty smears, since it was not unusual to find a nuclear shift in a person who was working eight hours a day and not feeling particularly ill. In most of these abnormal cases a recent cold, a sore throat, or chronic tonsil or sinus disease could be blamed. There were five cases in which no demonstrable reason for the shift could be found at that time.

In this paper, for purposes of clarity, the normal polymorphonuclear neutrophilic percentages are plotted in a broken-line graph, as shown in Fig. 1. This normal neutrophilic graph is repeated in all subsequent counts, to indicate the nuclear shift, whether to right or left as Arneth and Schilling have it. Actually, the shift is demonstrated to be clockwise (left) or counterclockwise (right) in these charts. Objection has been raised from time to time in the usage of the

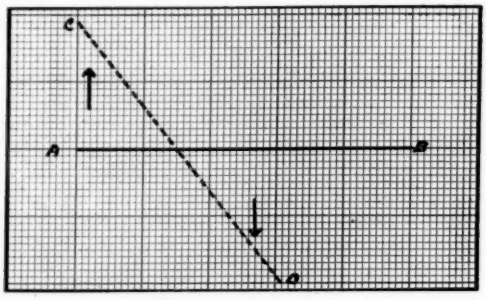

Fig. 2.—To illustrate the clockwise shift of the nuclear graph with infection. Line *AB* represents the normal neutrophilic status, and *CD* the relative position of the graph as the number of young neutrophiles increases.

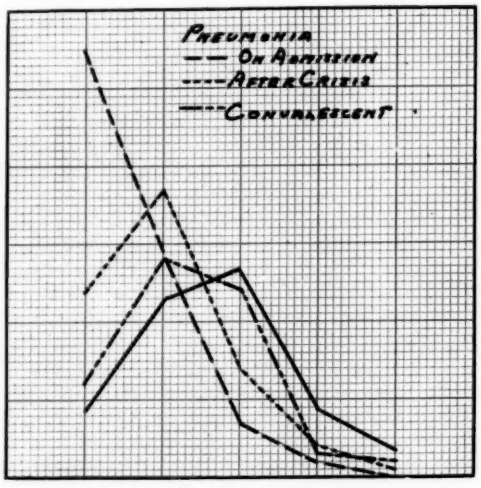

Fig. 3.—Lobar pneumonia. On admission, white blood count 19800, polymorphonuclears 93, small lymphocytes 4, large lymphocytes 3. After the crisis, white blood count 12600, polymorphonuclears 80, small lymphocytes 15, large lymphocytes 3, and monocytes 2. Convalescent, white blood count 7000, polymorphonuclears 70, small lymphocytes 21, large lymphocytes 7, monocyte 1, and eosinophile 1.

terms "right" or "left" shift. If the picture of the normal neutrophilic graph can be retained, as a balanced beam, with its fulcrum somewhere between the second and third classes, a more striking analogy can be gotten (Fig. 2). Thus it will be seen that with an increase in the blood stream of young cells, the balance beam will be thrown clockwise, and at the expense of the cells on the right of the fulcrum, which will therefore move down as the left of the beam moves up, and vice versa. Until a better term is introduced, I feel that a clockwise graph best indicates infection, or a neutrophilic shift to the left as interpreted by Schilling.

In the first group of cases (Figs. 3, 4, 5, 6, 7), are shown different varieties

of infection, all indicating a more or less pronounced clockwise shift of the nuclear graph, and, with recovery, or subsidence of the infection, a counter-clockwise movement back to normal. Thus, in Fig. 3 is pictured a case of pneumonia, with graphs drawn to indicate the nuclear shift at the height of the disease, following the crisis, and finally, just before discharge from the hospital. Fig. 4 illustrates a case of empyema, with the blood picture approaching normal

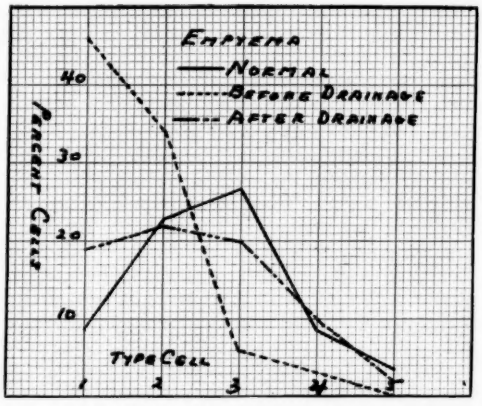

Fig. 4.—Empyema. Before drainage, white blood count 21000, polymorphonuclears 86, small lymphocytes 10, large lymphocytes 2, monocyte 1, eosinophile 1. Drainage was done at once, and after two weeks, white blood count 8000, polymorphonuclears 74, small lymphocytes 21, large lymphocytes 3, and monocytes 2.

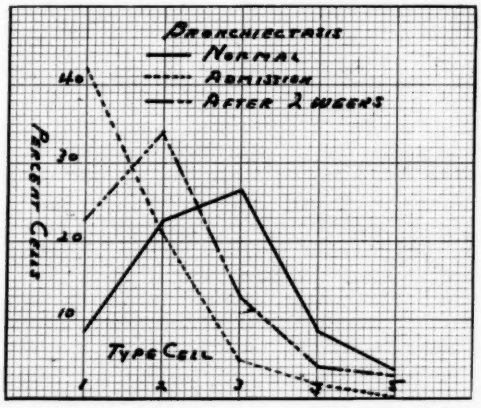

Fig. 5.—Bronchiectasis, with chronic bronchitis in a man fifty-four years of age. On admission, white blood count 10100, polymorphonuclears 70, small lymphocytes 19, large lymphocytes 6, monocyte 1, eosinophiles 3, basophile 1. After two weeks of postural drainage and inhalations, etc., white blood count 6800, polymorphonuclears 77, small lymphocytes 10, large lymphocytes 7, monocytes 2, eosinophiles 3, and basophile 1.

after drainage. Figs. 5, 6, and 7 are from relatively milder infections, none of them showing total white blood counts above 8000, yet all, before treatment, showing a clockwise movement of the nuclear graph. Evidence seems to indicate, as Farley et al. have pointed out, and which my own experience seems to confirm, that it is in this field where a careful nuclear differentiation will prove to be of very great help. In these cases presenting a normal total white blood count, and with the normal differential count showing nothing remarkable, it is often possible to show hidden infection by a shift of the nuclear graph. Cases

of chronic tonsillitis, oral sepsis, subacute arthritis (Fig. 9) or chronic arthritis (Fig. 8) almost uniformly will show a nuclear shift indicative of systemic infection, no matter how chronic or well hidden otherwise.

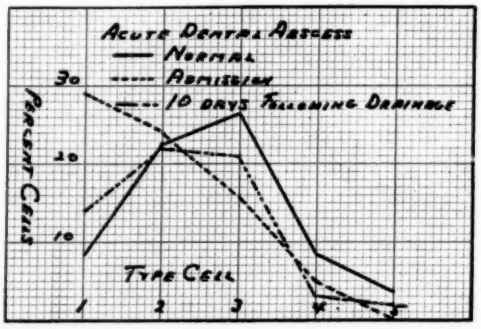

Fig. 6.—Acute dental abscess. On admission, white blood count 13200, polymorphonuclears 74, small lymphocytes 16, monocytes 3, and ecsinophile 1. Ten days following incision and drainage, white blood count 7400, polymorphonuclears 62, small lymphocytes 24, large lymphocytes 7, monocytes 3, eosinophiles 4.

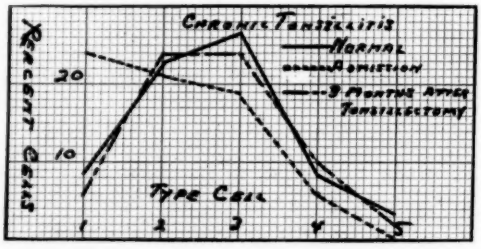

Fig. 7.—Chronic tonsillitis. On admission, white blood count 6600, polymorphonuclears 70, small lymphocytes 22, large lymphocytes 4, monocytes 2, eosinophile 1, basophile 1. Tonsillectomy was done, and the patient discharged from the hospital after recovery. Three months later, on returning to the hospital for check-up, white blood count 7000, polymorphonuclears 66, small lymphocytes 26, large lymphocytes 2, monocytes 3, eosinophiles 2, basophile 1.

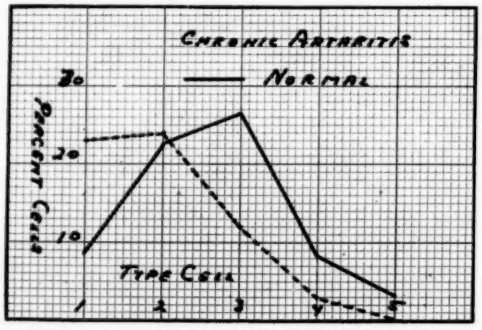

Fig. 8.—Chronic arthritis. In this patient all possible foci had been either checked and found negative, or else cleaned out, but the neutrophilic graph shows a distinct clockwise shift. Her symptoms at this admission were localized to one ankle, and the shift points to this as the infectious basis for neutrophilic stimulation. White blood count 7000, polymorphonuclears 62, small lymphocytes 30, large lymphocytes 2, monocytes 4, eosinophile 1, and basophile 1.

Obscure abdominal conditions, such as are pictured in Figs. 10 and 11, may well be mistaken for the more common acute intraabdominal lesions. In Fig. 10 is shown the nuclear curve from a case of ovarian carcinoma, which was producing confusing symptoms. In Fig. 11 is an ectopic pregnancy which could easily

have been mistaken for an acute salpingitis or appendicitis. In both cases, the absence of a marked nuclear shift was against an acute suppurative condition.

Patients presenting vague symptoms, those in whom the question always

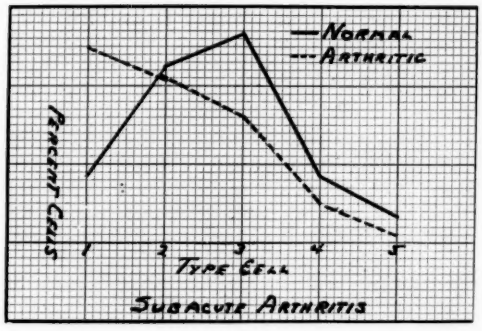

Fig. 9.—Subacute arthritis. Patient showed residual symptoms in one knee and in lumbar spine. White blood count 7500, polymorphonuclears 68, small lymphocytes 21, large lymphocytes 4, monocytes 4, and eosinophiles 2, and basophile 1.

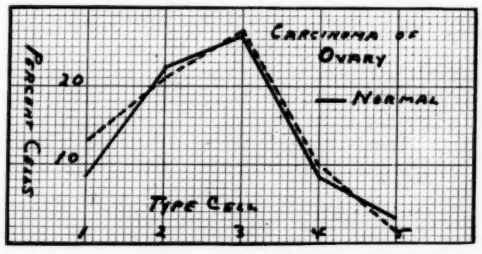

Fig. 10.—Carcinoma of ovary. White blood count 7600, polymorphonuclears 73, small lymphocytes 18, large lymphocytes 2, monocytes 4, and eosinophiles 3.

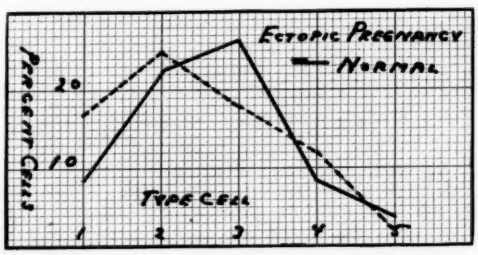

Fig. 11.—Ectopic pregnancy. Patient was a young white woman, twenty-four years of age, whose story and symptoms might have been either an acute appendicitis or an acute salpingitis. The neutrophilic graph shows her nuclear partition to be very little above normal. White blood count 13800, polymorphonuclears 74, small lymphocytes 17, large lymphocytes 3, monocytes 4, eosinophile 1, basophile 1.

arises as to whether the picture is functional or organic, offer an interesting field for the nuclear graph. In Figs. 12, 13, 14, and 15 are shown cases which seem to be of functional etiology. None of them offer any nuclear shift to indicate infection, and some support for the functional diagnosis is thereby gained.

That the blood forming tissues do anticipate demonstrable signs of infection, and begin, early, to indicate activity in relation to such an invasion is shown in Figs. 16 and 17. In Fig. 16 are neutrophilic graphs from three persons given the first immunizing dose of toxin antitoxin. Of the three, two showed slight rises

in total white blood cells, the third a leucopenia of 4200, and yet all three showed a distinct clockwise shift in the nuclear graph. In Fig. 17 are the nuclear curves of three women in the last week of pregnancy. All were afebrile, and, as far as a careful clinical examination showed, none of the three had any infection present. The total white blood counts ranged from 8200 to 9600. A nuclear shift in advance of any other sign of infection is thus demonstrated. It seems probable that this phenomenon takes place in many conditions, where the neutrophilic

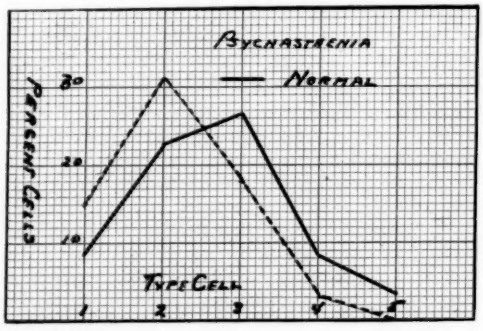

Fig. 12.—Psychasthenia, showing practically a normal nuclear partition. White blood count 7800, polymorphonuclears 67, small lymphocytes 28, large lymphocyte 1, monocytes 3, eosinophile 1.

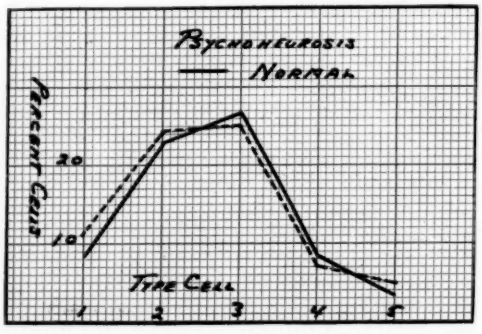

Fig. 13.—Psychoneurosis. This patient presented vague complaints, many of them, and the nuclear partition of the neutrophiles as pictured above points to a functional basis for his trouble. White blood count 7600, polymorphonuclears 72, small lymphocytes 18, large lymphocytes 5, monocytes 3, eosinophile 1, and basophile 1.

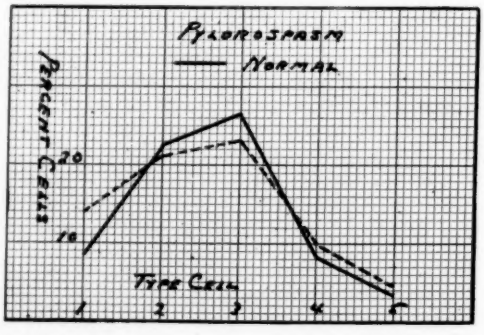

Fig. 14.—Pylorospasm. Differentiation here lay between a chronic peptic ulcer with obstruction or nervous pylorospasm. The neutrophilic partition points to a functional basis. White blood count 7700, polymorphonuclears 73, small lymphocytes 22, large lymphocytes 2, monocyte 1, eosinophile 1, and basophile 1.

cells increase to take care of an infection, real or potential, without clinical manifestations, other than might be shown by a neutrophilic count.

In Fig. 18 are pictured three cases of uncomplicated arterial hypertension. Of six cases available, these three were selected because no possible source of infection could be located. All three cases, however, with normal total white blood counts, showed a nuclear shift indicative of low grade infection. I do not wish to be placed in the position of attributing hypertension to chronic infection, but

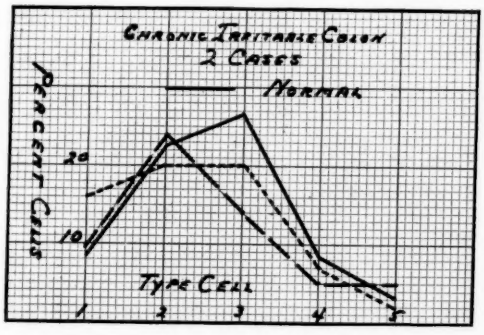

Fig. 15.—Chronic irritable colon (spastic colitis). Neither case shows a white blood count above 7400, and neither shows much evidence of infection in the neutrophilic nuclear partition.

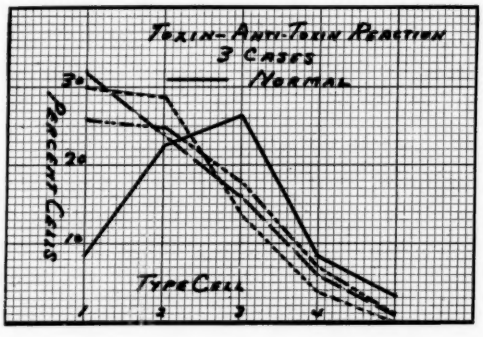

Fig. 16.—Toxin antitoxin reaction in three student nurses. None of the three developed any serious sequelae. The neutrophilic partition, however, points to a distinct neutropoietic stimulation.

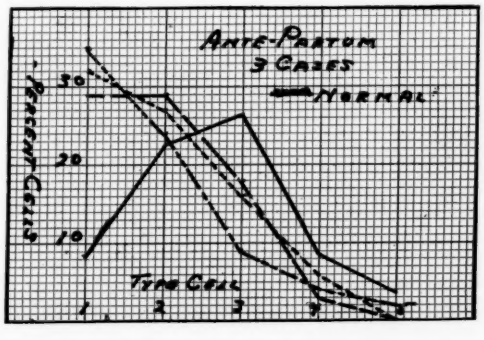

Fig. 17.—From three women in the last week of pregnancy. None of the three had any clinical sign of infection at this time, and all three passed through labor, delivery, and two weeks postpartum in the hospital without unusual symptoms.

merely point out that in three cases of hypertension, where infection could not be demonstrated, a nuclear curve was obtained which is probably indicative of infection.

Infectious mononucleosis is ordinarily interpreted as a disease process which stimulates primarily the lymphocytic centers, with a consequent increase in the large and small lymphocytes in the blood stream. Fig. 19 is presented merely to

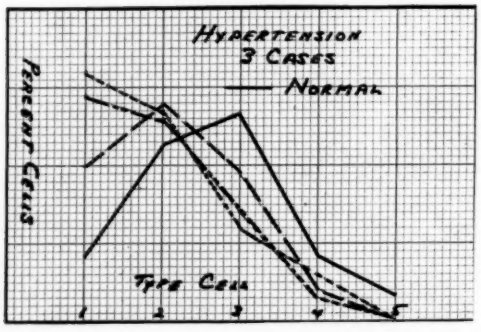

Fig. 18.—Hypertension, three cases. Total white blood count ranged from 7000 to 7400. No clinical signs of infection and no demonstrable foci.

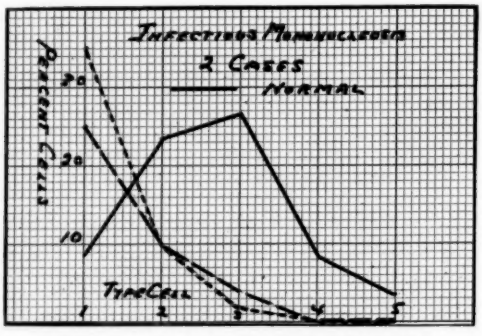

Fig. 19.—Infectious mononucleosis, two cases. Case 1, white blood count 4600, polymorphonuclears 39, small lymphocytes 17, large lymphocytes 41, and monocytes 3. Case 2, white blood count 11200, polymorphonuclears 47, small lymphocytes 15, large lymphocytes 32, and monocytes 6.

show that, even with but 20 to 40 per cent of polymorphonuclears in the stained smear, a strong nuclear shift is demonstrable. The mechanism is not clear, except that this is also an infectious condition, nor is the fact of a relative neutropenia in the face of marked neutropoietic activity discernible at this time.

Interesting phenomena occur in Figs. 20 and 21. In the former a case of ulcerative colitis is shown, where, week after week, a steady nuclear shift occurs, with few, if any, of the last or older three groups of polymorphonuclears being seen. As is shown, however, immediately after a transfusion, the old forms may be picked up in the stained smear, in small percentage. These are probably from donor's blood. In Fig. 21 is a case of hemorrhaging peptic ulcer, with a nuclear graph showing the status before and after transfusion, and also after development of a terminal bilateral suppurative parotitis. Although this case had carried a leucopenia of from 3000 to 5000 during twenty days in the hos-

pital, in spite of a nuclear shift as shown, on the development of the suppurative process the white blood cells shot to 18000 and the clockwise shift tremendously increased.

In Fig. 22 is shown a fatal pneumonia, with a relative leucopenia (9600). As indicated, the clockwise shift was profound, and, while it cannot be said that such a graph is indicative of overwhelming infection, it is nevertheless probable

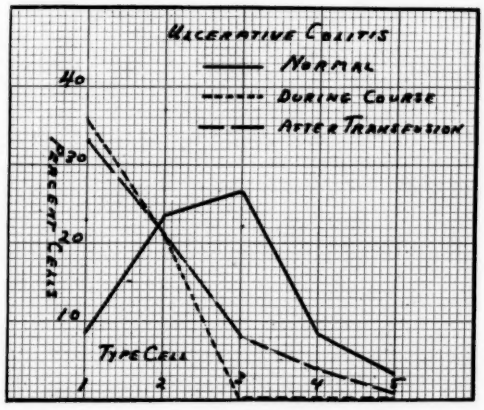

Fig. 20.—Ulcerative colitis. First count, taken two hours before transfusion, is sample of many taken during long hospitalization. Total white blood counts ranged from 8000 to 18000. First count: white blood count 12200, polymorphonuclears 67, small lymphocytes 24, large lymphocytes 4, monocytes 3, eosinophiles 2. Two hours after being given 500 cubic centimeters of citrated blood, the count was: white blood count 13600, polymorphonuclears 67, small lymphocytes 21, large lymphocytes 4, monocytes 6, eosinophiles 2.

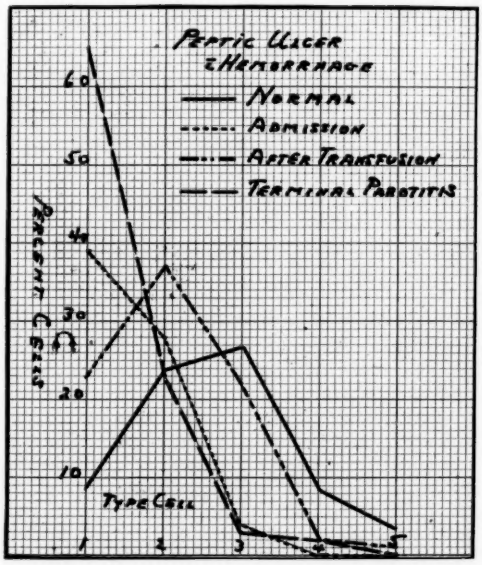

Fig. 21.—Peptic ulcer with repeated hemorrhages. Count taken two hours before transfusion shows white blood count 4200, polymorphonuclears 71, small lymphocytes 23, large lymphocytes 5, eosinophile 1. Two hours following transfusion of 400 cubic centimeters citrated blood, the count was: white blood count 5600, polymorphonuclears 83, small lymphocytes 14, large lymphocytes 2, and monocyte 1. Six days later the patient developed a bilateral suppurative parotitis, and at this time the count was, white blood count 18000, polymorphonuclears 93, small lymphocytes 5, large lymphocytes 2.

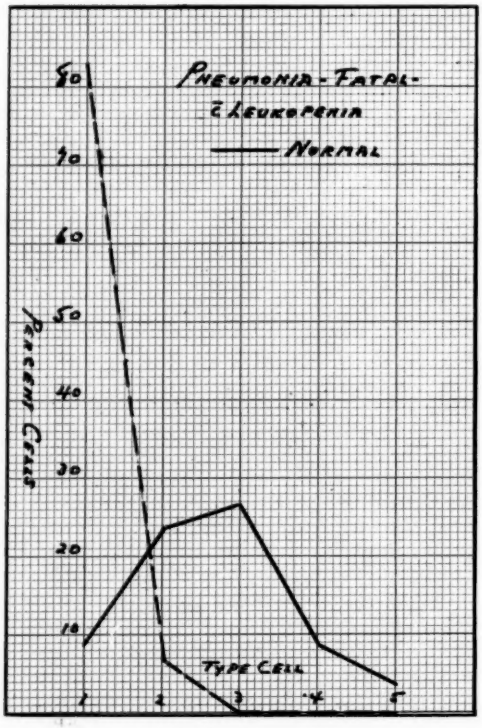

Fig. 22.—Type I pneumonia in a man of sixty years. Admitted to hospital on fourth day of illness. White blood count 9600, polymorphonuclears 90, small lymphocytes 6, large lymphocytes 4. Patient died twelve hours later.

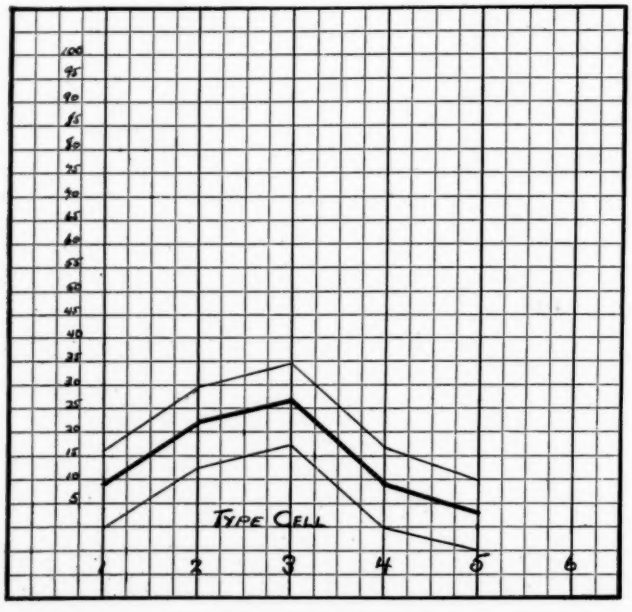

Fig. 23.—Suggested form for routine plotting of neutrophilic partition.

that a shift to such a degree is to be expected in the more profound and grave types of infection.

Fig. 23 shows a form of chart which may be adapted to clinical use. On it are shown, in heavy black line, the average normal figures, while above and below are lighter lines which might be said to be the upper and lower limits of normal in each cell group.

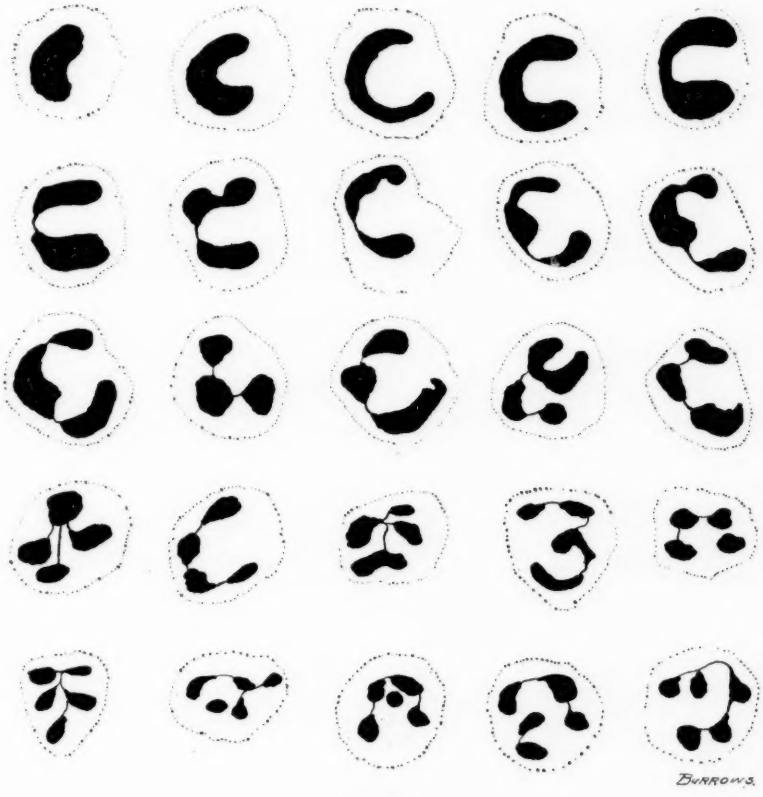

Fig. 24.

SUMMARY

1. Recognition is given to pioneers in the field of differential neutrophilic counts.

2. A new series of differential neutrophilic counts is presented, with average normal figures for each of the common cell-types in the stained smear.

3. A new method of graphically presenting the nuclear shift is proposed.

4. Graphs are shown from various conditions in support of the thesis as cited.

5. It must be stated again that this paper is not an attempt to cover the field. The way is only just opened. It is hoped that in future papers the blood picture in various diseases will be more thoroughly investigated in the light of present day knowledge as to the importance of the complete neutrophilic differential count and its graphic illustration.

CONCLUSIONS

1. The normal number of nonfilament or one-lobed polymorphonuclear neutrophilic leucocytes in the blood stream is from 6.08 to 9.2 per cent and in the series presented, 8.45 per cent. These figures are average.

2. The normal number of the older neutrophiles is determined to be as follows:

2 lobes—22.99%
3 lobes—26.7 %
4 lobes— 8.7 %
5 lobes— 3.21%

3. Infectious processes will cause a definite shift in the neutrophiles, the extent depending on the severity of the infection.

4. This shift takes place whether with or without a rise in the total number of circulating white blood cells.

5. There is reason to believe that hidden or focal infection can be demonstrated by this method.

6. There is also reason to believe that acute purulent conditions may be better differentiated from other conditions causing like symptoms.

7. Evidence is presented to show that functional conditions may be better differentiated from organic conditions.

Acknowledgment is made for valuable advice and cooperation in the preparation of this paper to Dr. F. J. Sladen and Dr. F. W. Hartman.

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THE AGE OF THE LEUCOCYTE IN RELATION TO INFECTION*

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THE leucocytes, or white cells, of normal blood are classified as neutrophiles, eosinophiles, basophiles, monocytes, and lymphocytes. The changes in absolute and relative numbers of these various groups of cells, as a result of infection or other disturbance, are not within the scope of this review. Such numerical changes constitute the ordinary data of absolute and relative leucocytosis and leucopenia, which have received the major share of attention in clinical hematology for many years.

Study of correlated qualitative changes within the members of the several white cell groups dates back to Ehrlich, but has not found widespread clinical application until recent times.

Arneth's[1] pioneer researches (1904) constitute the ground-work and at the same time mark the point of departure for subsequent developments. Arneth's hypothesis in regard to the neutrophile's response to infection is based on two correlated major premises: (1) that young neutrophiles are supplied in abnormally increased numbers to the blood stream as a fundamental defense mechanism against infection, and (2) that the age of neutrophiles varies directly with, and can be measured in terms of, nuclear polymorphism or lobulation. Arneth extended his general theory to include other white cells,[2] but the major interest has centered about the neutrophiles.

THE MEANING OF "YOUTH" AND "MATURITY" OF LEUCOCYTES

When hematologists designate a given circulating leucocyte as "young" or "mature" or "senile" there are a number of conditional assumptions implied, and the meaning of such age-judgments may vary considerably. A statement of "age" presupposes knowledge of a beginning. It seems fair to suggest that, when dealing with circulating leucocytes, the "beginning" is thought of vaguely in terms of either moment of entry into the blood stream, or moment of definitive differentiation in the formative center, or a combination of both. Thus a myelocyte in the peripheral blood is a "young" form because, first, it is assumed that a living myelocyte quickly matures into a neutrophile leucocyte after liberation from the formative tissue, and second, its morphology identifies it with the early granule-containing cell in the formative tissue. Neither of these assumptions, however, need necessarily be valid.

A myelocyte in the blood stream might conceivably be a relatively stable and long-lived individual analogous to a "dormant" cancer cell or a bacterial "spore." If this should be true its presence in the blood stream would lose all significance, for purposes of age judgments, from this standpoint. Viewed from another angle, the "aging process" may produce varying definitive stages with different "spans" of life under varying conditions. No one knows how long

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a given myelocyte may remain undifferentiated, growing "older by the clock," before it "maturates" or "divides" or before it enters the blood stream. No one knows with certainty the "normal life-span" of the neutrophile in the blood. It is estimated in terms of "hours" by some,[3] "days" by others,[4] and "weeks" by others.[5]

In spite of these theoretical uncertainties, the working concept of "youth" and "age" judgments of leucocytes is that *a leucocyte is young in all senses of the word when it looks and behaves like its embryologically established (or assumed) progenitor in the leucopoietic centers.*

GENERAL CRITERIA OF YOUTH OF LEUCOCYTES

The morphologic evidences of immaturity of leucocytes may be conveniently considered under (a) nuclear and (b) cytoplasmic.

NUCLEAR EVIDENCES OF IMMATURITY

1. Mitosis and amitosis are universally admitted as certain proof of immaturity. This is especially true of mitosis, which cannot be simulated, by cell rupture or other artifact, as easily as amitosis. Both forms of cell division have been observed in practically all of the immature leucocytes in the formative centers, as well as in the blood stream in leucemias. Maximow[6] insists that amitosis does not occur in lymphocytic cells, but other observers disagree.[7] In the "granular series," cell division is demonstrable in the myeloblast, the promyelocyte, and the myelocyte, but not thereafter (i. e. not in metamyelocytes "band forms" or polymorphonuclear neutrophiles). In the lymphatic series Maximow (loc. cit. p. 329) describes mitotic figures in large and in medium sized lymphocytes and states that small lymphocytes do not divide. Sabin emphasizes amitosis as the usual form of myeloblastic division.

In clinical hematology (except in study of the leucemias) *the occurrence of peripheral leucocytic mitosis and amitosis is of little practical value in estimating age, for the reason that almost all circulating leucocytes are relatively "mature" and are incapable of further cell-division.*

2. Nucleoli within the nucleus of leucocytes constitute valid proof of immaturity. The actual demonstration of nucleoli is, however, not always an easy and certain procedure. In the granular series the myeloblast nucleus is, according to all observers, characterized by the presence of two to six nucleoli. The same is true of the promyelocyte. The myelocyte nucleus, however, presents uncertainty. Downey[8] states that the myelocyte proper and its descendants (metamyelocytes, polymorphonuclear neutrophiles, eosinophiles and basophiles) are totally devoid of nucleoli. Bunting (loc. cit. p. 405) and Schilling,[9] on the other hand, find nucleoli in myelocytes and metamyelocytes, and Naegeli[10] agrees that occasionally nucleoli may be seen in myelocytes. All authorities concur in the opinion that ordinary polymorphonuclear neutrophiles have no demonstrable nucleoli. Bunting finds, however, (loc. cit. p. 405) that special staining may bring out nucleoli in the "young" polymorphonuclear neutrophile, eosinophile, and basophile cells of the blood.

In the lymphocyte series the lymphoblast nucleus is unanimously accorded nucleoli, although authorities differ as to the characteristic numbers (1 to 2

nucleoli according to Naegeli and Schilling, up to 5 or 6 according to Schridde and Butterfield). The large "young" lymphocyte of the blood may have nucleoli (Schilling, loc. cit. p. 131) but mature lymphocytes have none (Downey, loc. cit. p. 375).

The monoblast (Naegeli) may contain nucleoli. Concerning monocytes proper there is some disagreement, no nucleoli according to Downey (p. 376); occasionally present according to Schilling (p. 132) and Naegeli (p. 143) who emphasizes the importance of vital stains in this connection.

From this summary it is clear that the *status of nucleoli in the several leucocyte types is hardly definite enough to offer much help in clinical hematology except in research work and in the study of leucemias.*

3. Chromatin structure of the nucleus is recognized, in stained preparations, as a most significant criterion of age. Young leucocytes possess vesicular nuclei formed like a net built of fine basic staining threads (Naegeli p. 178). The nucleus of older leucocytes is more coarsely constructed with heavier bands and pieces of deeper staining basic chromatin. Older nuclei also show either a tendency to pycknosis or a tendency to sharp differentiation of basi and oxychromatin, which are features lacking in young nuclei. The nuclear membrane is thin and indistinct in very young cells and is thicker and clearer in older cells, with a submembranous condensation of basic chromatin that emphasizes it all the more.

The chromatin structure of the nucleus of leucocytes is considered by many hematologists to be the soundest and most useful general criterion of age.

4. Nuclear polymorphism or lobulation as an index of leucocyte maturity constitutes the major premise of Arneth's theory. All observers are agreed that the nuclei of primitive white cells are round or oval and that the nuclei of most mature leucocytes (lymphocytes excepted) show more or less indentation, lobulation, and polymorphism. Arneth holds, for the neutrophiles particularly and for other leucocytes to a certain extent, that age duration and multiplicity of nuclear lobulations are directly and mathematically correlated.

This fundamental position of Arneth's is attacked by certain other hematologists along several lines. For example Naegeli says (loc. cit. p. 178): "entirely erroneous is the procedure of Arneth by which the age of the cell is estimated from the degree of segmentation of its nucleus. There are many cells most certainly young as judged by nuclear structure which are markedly segmented, especially is this true of monocytes but also of neutrophiles." Bunting says (loc. cit. p. 405): "leucocytes with basophilic protoplasm, loosely woven nuclei and even with basophilic granules among the neutrophilic granules, and thus obviously young cells, may show as many lobes to the nucleus as cells evidently senile."

The practical application and developments of Arneth's doctrines will be discussed below, together with their difficulties and limitations. The above remarks indicate the somewhat *uncertain status of the basic evidence of Arneth's contention.*

CYTOPLASTIC EVIDENCES OF IMMATURITY

1. Mitochondria, demonstrable by supravital stains (e. g. Janus green), are present in all young white cells.[11] These structures seem to diminish in number

as the cell matures. Sabin[12] finds that, as the myeloblast grows older, the multitudinous mitochondria (staining green in the neutral red Janus green mixture) become fewer and the specific (red staining) granules begin to make their appearance. In the mature myelocyte there are very few mitochondria and more "specific" granules, and in the mature polymorphonuclear neutrophile there are no mitochondria at all. Similarly according to Sabin and her coworkers, the monoblast with many (green staining) mitochondria matures into the large mononuclear type of monocyte with fewer mitochondria and this in turn into the "transitional" type of monocyte with but very few mitochondria (and a fully developed neutral red rosette). A similar diminution of mitochondria is described in the transition of the lymphoblast to the lymphocyte.

The practical uses of "supravital" stains in clinical hematology have been directed more toward attempts at differentiation of cell types than toward estimation of cell maturity. *It would seem that a careful study of leucocytes by supravital technic correlated with the ordinary dry smear stains (Wright's, etc.) in each instance might yield valuable evidence regarding the validity of our ordinary morphologic criteria of youth and maturity.**

2. Cytoplasmic basophilia is a *generally accepted indication of relative immaturity of circulating leucocytes.*

3. The centrosome and the Golgi apparatus, when present, are probably evidences of relative immaturity. The demonstration by special staining methods of these structures has, as yet, no place in clinical hematology.

4. Cell size is of little or no value as a criterion of age except in this very general way: the progenitors of the blood leucocytes tend to become smaller as they mature (Naegeli p. 179). Young neutrophiles are said to be larger than mature ones (Schilling p. 128) but the "macropolycyte" of Cooke and the large "pernicious anemia neutrophile" would seem to be obvious exceptions to this general statement. In regard to lymphocytes there is also no established correlation between cell size and age. The large lymphocytes are not necessarily younger than the small and medium sized lymphocytes (Naegeli p. 135; Maximow p. 329, 358). Finally the stem cells themselves are found to present marked variation in size in the formative centers and their descendants in the blood stream are subjected not only to these inherent developmental variables but also to extraneous factors such as osmosis and hydropic changes, all of which must have an influence on "size."

5. Cytoplasmic granulation (as it appears in polychrome stains) in the neutrophile series of leucocytes presents features of importance in relation to the age of these cells. Young neutrophiles have more basic staining granules scattered in with the neutrophilic granules than do the mature forms. In the latter the specific granulation of the cytoplasm is purely neutrophilic or even slightly acidophilic in quality.

This tendency to basic granulation of young neutrophiles is recognized as a most significant criterion of immaturity by most observers.

6. Amoeboid activity and phagocytic activity of circulating leucocytes in warm-stage preparations are acceptable evidence of vitality and functional efficiency but have no direct bearing on the problem of age. Obviously dead and

*A correlation study of this sort is being planned in this clinic in collaboration with Byron E. Hall.

dying cells will not exhibit these phenomena but not all dead cells are old cells, especially in toxic and diseased stages. Furthermore young leucocytes are not necessarily more active than older cells of the same type. It is recognized, for example, that myeloblasts and myelocytes show much less evidence of these sorts of activity than do mature neutrophiles.

The Age of Leucocytes (Other Than Neutrophiles) in Relation to Infection.—As previously indicated the chief interest in the age of leucocytes in relation to infection centers around the neutrophile. Arneth has attempted to bring the other white cells into line with his theories but this attempt has met with little or no recognition of success (see Schilling, loc. cit. p. 147). We have reviewed briefly the general criteria that are applicable. The difficulties are obvious.

These difficulties become still greater when one digs deeper into the mass of data at hand. "In cases of monocytosis, transitions between lymphocytes and monocytes in the blood are fairly numerous" (Maximow, loc. cit. p. 463). Small lymphocytes do not divide but "may change over into large lymphocytes or monocytes or plasma cells" (Maximow, p. 329, 349, 358). Plasma cells are identical with Türk irritation cells and are thought to arise from lymphocytes by Maximow, from myeloblasts by Downey (loc. cit. p. 377) and from both types of stem cell by Naegeli (loc. cit. p. 176-177). The so-called Rieder cell is a circulating myeloblast with indented nucleus according to Downey, who admits the great difficulty of differentiating it from monocytes. The "Rieder type" is of lymphoblastic origin according to Schilling (p. 135). This observer notes the occasional "incongruity in the maturity of nucleus and protoplasm" in various types of leucocytes. We have already indicated the impropriety of correlating the large and small lymphocyte with the young and old lymphocyte. Concerning eosinophilic leucocytes there is little or nothing of practical value that is known in this connection. We have personally studied the blood of a patient with eosinophilic hyperleucocytosis[13] in which the striking feature was the presence of huge numbers of perfectly normal looking "adult" eosinophiles with polymorphous nuclei and no eosinophilic myelocytes or other evidence of "shift to the left" of the eosinophile formula, although at necropsy this patient's bone marrow was found packed with eosinophilic myelocytes. We have on the other hand seen mononuclear eosinophiles (young forms?) in the blood of a case of trichiniasis which recovered.[14]

The Age of the Neutrophile in Relation to Infection.—The Arneth "count" of neutrophile nuclear lobulations, and to a certain extent the modifications of this procedure (the Schilling Hemogram, the polynuclear count of Cooke and Ponder, the nonfilament neutrophile count of Farley, etc.) are open to criticism and objection from several standpoints: (1) embryologic, morphologic, and physiologic, and (2) technical and utilitarian.

Before considering the subject from these angles, a brief review is necessary of the theory and practice of Arneth and his successors.

The Arneth Count.—The neutrophile cells of normal blood, according to Arneth, are classifiable into five groups. Class I (5 per cent of neutrophiles) are cells whose nucleus is round, oval, or indented but not truly lobulated. Class II (35 per cent of neutrophiles) are cells with two lobed nuclei. Class III (41 per cent of neutrophiles) are cells with three lobed nuclei. Class IV (17 per

cent) are neutrophiles with four lobed nuclei, and Class V (2 per cent) are neutrophiles with five lobed nuclei. Arneth makes numerous subdivisions within each class so that in all he recognizes twenty distinct types of circulating neutrophiles. He believes that increased nuclear lobulation is direct evidence of increased age from beginning to end. He further believes, as a direct result of his laborious researches, that a "shift to the left" in the neutrophile formula occurs as a result of infection. By this he means a relative increase in the "young" neutrophiles belonging in the "left hand" columns of cells (Classes I and II). According to Arneth a proper study of the "nuclear shift" constitutes a more sensitive index of the presence of and trend of infection than does the ordinary "total leucocyte and differential count" usually employed.

As to the mechanism of leucocytosis and shift to the left in infection, Arneth contends that this represents a disturbance of the normal balance between peripheral leucocyte destruction and central (secondary) new cell production.

The Schilling Hemogram.—Victor Schilling, recognizing the merit of Arneth's (overcomplex) procedure, made certain important modifications. He felt that Arneth had gone too far in correlating increasing maturity with increasing nuclear lobulation. Schilling therefore grouped all the cells of Arneth's Classes II to V inclusive into one class as "segment-nuclear" (mature) neutrophiles (63 per cent of the normal total leucocyte count; 94 per cent of the normal neutrophiles). The other neutrophilic granular cells of the blood are classified by Schilling as myelocytes (absent in normal blood); "rod nuclears" or "band forms" corresponding to mature metamyelocytes (4 per cent of the normal total leucocyte count; 6 per cent of the normal neutrophiles); "young" or "juvenile" neutrophiles, corresponding to Pappenheim's young metamyelocytes and differing from the "rod nuclears" chiefly by the sausage or bean-shaped character of the nucleus with nucleoli in contrast to the rod-shaped nucleus without nucleoli; (these juvenile forms are designated by Schilling as "rarely occurring" in normal blood but sometimes present in numbers up to 1 per cent of the total leucocytes); a special degenerative "stab" or staff form of nonsegment nuclear neutrophile which is according to Schilling a "neutrophile matured without segmentation." These "degenerated rod nuclears" or "stab" forms are absent from normal blood and are of especial significance in Schilling's theory and practice, in that they represent a distinct contradiction of Arneth's fundamental theory, and play a major rôle in Schilling's hypothesis of a "degenerative shift to the left."

Schilling's hemogram includes a tabulation of (1) myelocytes, (2) juvenile neutrophiles (young metamyelocytes), (3) "rod-nuclears or "band forms" (old metamyelocytes), (4) "stab" nuclears (a degenerative nonsegment nuclear of special type), and (5) segment-nuclear ("adult") neutrophiles together with (6) the usual differential count of lymphocytes, monocytes and eosinophiles. Each of these items in the hemogram is expressed in percentage of total leucocytes (and not, as Arneth had done, in percentage of neutrophiles).

Cooke's Polynuclear Count.—Arneth's criteria for separating the number of nuclear lobes were not specific. Schilling gave more attention to this difficulty, but since he grouped all neutrophiles with more than one lobe into one class (of "segment-nuclears"), he was not greatly concerned about the question. Cooke

(1914) regrouped the "polymorphs" into five classes, analogous to Arneth's classes, but more strictly defined[15] by the following morphologic dictum: "If there is any band of nuclear tissue except a chromatin filament connecting the different parts of a nucleus, that nucleus cannot, for the purposes of the count, be said to be divided."

On this basis Cooke's Classes III, IV, and V are practically identical with Arneth's Classes III, IV, and V. Cooke's Class I, however, for some curious reason does not include the myelocyte or the young metamyelocyte. It includes all of the cells of Arneth's Class I, except myelocytes and metamyelocytes and some of Arneth's Class II (i. e. those with twisted nuclei showing a central construction but not a true "filament"). Cooke's Class I includes all of Schilling's nonsegment neutrophiles (except the myelocyte and young metamyelocyte). Cooke's Class II is identical with Arneth's Class II, except for a few nonfilament nuclears with "pseudo-bilobulation" which Cooke's criterion makes him classify as "undivided" (i. e. as belonging in Class I). In normal blood the average number of Class I cells is 10 per cent of the neutrophiles (or about 7 per cent of the total leucocytes). Cooke's findings and views concerning the "macropolycyte" are outside the scope of this review, except that they constitute a partial break in his otherwise whole hearted allegiance to Arneth's general hypothesis.

"The Simplified Arneth Count" of Pons and Krumbhaar.—Pons and Krumbhaar (1924) adopted Cooke's criterion of the "filament" but suggested[16] that the essential feature of the neutrophile count could be maintained by a three fold classification into (1) metamyelocytes, (2) nonsegmented band forms, and (3) segmented forms with filament connections between the lobes.

The Filament and Nonfilament Polymorphonuclear Neutrophile Count.—Farley[17] and his coworkers (1930) using Cooke's criterion of the filament, divide the neutrophiles into nonfilament forms (practically identical with Cooke's Class I) and filament forms (Cooke's Classes II to V inclusive) and report them separately as components in the ordinary complete differential count. These workers report an average of 9 per cent nonfilament neutrophiles in a study of 100 normal individuals and set 16 per cent as the upper limit of normal. These figures represent percentage of total leucocytes (not percentage of neutrophiles).

Our own procedure (since 1928) also based on Cooke's criterion, has been to include in the routine differential count, a bracket, after the figure for the polymorphonuclear neutrophile percentage, into which are set down the percentages of "old" and "young" forms seen in making the differential count of 100 white cells. A "normal differential" would be as follows: Polymorphonuclear neutrophiles 62 per cent (56 per cent "old"; 6 per cent "young"); lymphocytes 29 per cent; monocytes 6 per cent; eosinophiles 3 per cent; basophiles 0; myelocytes 0. A "typical" count in very severe infection would be: Polymorphonuclear neutrophiles 88 per cent (45 per cent "old"; 43 per cent "young"); lymphocytes 9 per cent; monocytes 2 per cent; eosinophiles 0; basophiles 0; myelocytes 1 per cent.

In our procedure the designation of "young" forms comprises all nonfilament neutrophiles (i. e. all of Cooke's Class I cells) and all metamyelocytes as well (i. e. all of Schilling's "juvenile forms," "rod-nuclears," and "stab"

forms). Farley's classification is identical and serves the added good purpose of employing a more exact descriptive terminology (i. e. filament and nonfilament instead of "young" and "old").

Certain refinements such as the "double shift" and the "metaplastic shift" of the nuclear index are not encompassed by these simplified procedures.

Embryologic, Morphologic, and Physiologic Objections to the Arneth Hypothesis and Its Modifications.—Arneth's correlation of neutrophile age-increase with increase of nuclear lobulations has been criticized by many observers. The primary difficulty of meaning of "age concepts" has been mentioned. The varying estimates of "duration of life" of the circulating neutrophile have been referred to. The lack of correlation between cell age and nuclear segmentation is emphasized by Naegeli, Bunting and others. Schilling's concept of the degenerative "stab" nuclear must be classed as evidence against Arneth's correlation hypothesis. In the field of comparative (animal) leucocyte morphology there is some evidence that nuclear lobulation may vary in different species. Personal observation of the blood of the young white rat indicates that the great majority of neutrophiles are either nonfilament or single filament forms (i. e. Class I and II of Cooke) although the nucleus may be quite tortuous. The neutrophile of the monkey, macacus rhesus, is reported by Krumbhaar[18] and Hall[7] as characteristically hypersegmented. Hall's drawings show typical neutrophiles with nuclei composed of 5 to 6 lobes connected by very fine chromatin filaments. Krumbhaar and Musser state that neutrophiles containing nuclei "with 10, 12, and 15 lobes were frequently encountered." These observations suggest that "age" is certainly not the only factor concerned in nuclear segmentation.

The several modifications and "simplifications" of Arneth's complex procedure are free from many of its fundamental objections. The "modificationists" (except Cooke) either frankly disagree with Arneth concerning his age correlations in groups beyond Class I, or dodge the issue entirely. Cooke, however, and later Cooke and Ponder[5] go the whole way with Arneth in linking up nuclear segmentation with cell maturity. All are agreed in the general proposition that the nuclear "shift to the left" in the neutrophile formula reflects an increase in relatively young forms and constitutes an important method of study.

Cooke and Ponder have presented certain experimental evidence, which if extended and confirmed, would strongly support Arneth's major premise. The reader is referred to the monograph[5] of these authors for details. It is interesting to note that Cooke and Ponder estimate "the whole length of life of a polymorph in the blood stream" at "about three weeks" (p. 24 of their monograph). This contrasts sharply with the statement of Bunting that "the life of the neutrophile within the blood is measured by days and even almost by hours." Both of these statements are somewhat at variance with the usual present day assumption of a "four-day life span."

It is of further interest to note that, contrary to Cooke and Ponder the findings of Isaacs[19] suggest "that when immature cells enter the blood stream they are eliminated as such and do not mature in the peripheral circulation."

From the physiologic standpoint the Arneth hypothesis presents certain difficulties. The efficiency of young neutrophiles as compared to mature neutro-

philes in defense against infection is a highly controversial subject which is beyond the scope of this presentation (see Cooke and Ponder, p. 46-49). A corollary of this difficulty concerns the question of prognosis.

Technical and Utilitarian Objections.—Many of the technical (morphologic) difficulties of the original Arneth procedure are minimized by the application of Cooke's criterion of the filament. There are other difficulties, however, which remain inherent in the method, regardless of "simplifications." For example we have seen an experienced hematologic technician mistake a large number of monocytes (of the "transitional type") for supposed young (band-form) neutrophiles. We have not infrequently seen mononuclear cells that might be classified as metamyelocytes by one observer and "Rieder cells" by another and monocytes by another. Occasionally it is difficult to decide whether a given nucleus is of filament or nonfilament type.

As to the best technic of stain there is sharp divergence of opinion. Schilling, Cooke and Ponder, and others recommend smears of blood on glass slides, and the employment of Giemsa stain. These authors condemn the use of Wright's, Lieshman's and Romanowsky's stains as "not satisfactory for the purposes of the Arneth count." Piney[20] recommends cover-slip preparations with modified Giemsa stain. Farley and his coworkers prefer Wright's stain and cover-slip preparations. In this country it seems fair to state that the fate of the neutrophile count in clinical hematology will be decided on the basis of Wright's stain (or a similar Romanowsky modification).

The utility of these procedures in clinical practice depends upon (1) the relative simplicity of execution, (2) the accuracy of results, and (3) the helpfulness in diagnosis, prognosis, and treatment.

The difficulties of execution of the simplified procedures are not great, and fairly accurate results may usually be obtained in the hands of experienced workers. Our own observations, however, would indicate that the "mean error" of the method is considerably greater, and the "personal equation factor" much more important than is admitted by some enthusiasts (e. g. see Cooke and Ponder, p. 8, 14). This is particularly true in many cases of serious infection in which toxic and degenerative changes in the leucocytes are profound.

The diagnostic utility of these methods is very limited. Such study may *occasionally* disclose the presence of an unsuspected infection or other obscure disturbance of organic health. It is of practically no value in the differential diagnosis of infections. It sometimes is helpful in calling attention to the development of untoward complications in the course of infections.

The prognostic implications of the "nuclear shift" in infections are of considerable interest. All observers agree that no deductions as to prognosis can be made except on the basis of repeated observations. The fundamental problem is this: how much of a "shift to the left" and what kind of shifts are to be viewed as "satisfactory" or "normally favorable" evidence of response to infection? There is, in our opinion, no easy answer or ready formula or infallible index. The unknown individual "constitutional" peculiarities[21] which make one person exhibit high fever or marked "leucocytosis with shift to the left" from some apparently trivial infection, may in another person behave quite differently under apparently similar circumstances. In our experience[22] the

state of pregnancy and puerperium is one in which infections are particularly prone to produce exaggerated leucocyte responses. The same seems to be true of certain infections in infancy. From another standpoint, some infections seem to cause intense stimulation of one kind of leucocyte, other infections another kind of leucocyte, and still others cause little or no leucocyte stimulation.

Given a knowledge of the type of infection present, the age and general status of the patient, and the usual or "normal" leucocyte response to the infection, it is then quite possible to obtain, from accurate serial observations of the changes in the "nuclear index," the "staff count" or the "nonfilament neutrophile count" some information of prognostic value.

SUMMARY AND CONCLUSIONS

1. The absolute age of the different leucocytes in health, their normal "life span" in the blood stream, and the duration of the several "stages" in their normal life cycles, are all uncertain.

2. The absolute age of leucocytes in relation to infection is also uncertain.

3. The relative age of neutrophile leucocytes in the blood stream is partly measurable, in terms of nuclear segmentation, to this limited extent: nonsegment (nonfilament) nuclears are for the most part "younger" than segmented (filament) nuclears.

4. In states of infection the proportion of relatively young forms of neutrophiles is *usually* (but not always) increased above that level which is more or less characteristic of health.

5. This increase of nonfilament forms in the differential count may *occasionally* be of some diagnostic importance in that it *may* direct attention to the existence of occult infection or to the development of an unsuspected "complication." Infection, however, is not the only cause of a "shift to the left."

6. The magnitude of the increase (i. e. the degree of "shift to the left") and the character of the "young forms" involved in the increase (i. e. the type of "shift") may be of some prognostic value when repeated observations are made.

7. The prognostic value of such observations is far from absolute and the "information" thus obtained is to be considered in proper relation to all the other available evidence.

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A STUDY OF THE WHITE AND DIFFERENTIAL COUNTS IN SIX UNSELECTED CASES OF INOCULATION MALARIA*

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SURPRISINGLY little has been reported on the leucocyte and differential counts in malaria, most of the work being confined to investigation of the red blood cells. It was thought that a study of these cells might prove of value in inoculation malaria, as used in the treatment of paresis, both from a clinical standpoint, and as a means of determining whether or not this inoculation malaria differs from clinical tertian malaria. It was also decided to study the so-called nonfilament count, with the hope of gaining more information, particularly in regard to the severity of the fever.

Craig[1] reports "As regards the white corpuscles, in general, the reduction in their number corresponds with that of the red cells. During the paroxysm there is often a leucocytosis, while between the paroxysms the leucocytes are markedly reduced in number. This is, in general, true of all forms of malarial fever, but in some cases of fatal pernicious malaria leucocytosis is observed. Much attention has been paid to a relative increase in the mononuclear leucocytes as of diagnostic importance in malarial infection. While probably in a majority of instances there is a considerable increase in this type of cell, it has not been the writer's experience that very much weight can be given in diagnosis to a mononuclear increase, as it occurs in so many conditions not malarial in nature." Alteration of the leucocytes is said by one observer[2] to be the first thing observed following inoculation. This alteration preceded the fever, and even the appearance of the parasite in the blood, or enlargement of the spleeen. Leucocytosis is reported by Stitt[4] to occur only in malignant forms, or in the presence

*From the Cleveland Clinic.

of complications. Emerson[5] and Thompson[6] report a leucocytosis at the time of rigor, with a moderate leucopenia during the apyrexial period. Thompson[6] reports, in addition, a leucopenia when the parasites are numerous, a rise when they are few, and a marked leucocytosis when they cannot be detected. Morrison[7] notes that the leucocytes are below normal during the febrile periods, and above normal after these periods.

As regards the differential count, the most constant and persistent finding in malaria has been an increase in the monocytes. This monocytosis is said to be pronounced in the apyrexial periods[5, 8, 9] and least marked during the febrile periods, and is the inverse of the temperature curve.[10, 7] When monocytosis is late in appearing it is preceded by a high lymphocytosis.[2] Morris[8] reports a monocytosis during the apyrexial periods as high as 90 per cent, persisting some months after the fever has subsided. This monocytosis is said by many to be of value in diagnosis. Bohn[11] believes that the blood picture is so characteristic as to be diagnostic even in the absence of the parasites. Swan[12] does not believe that a large mononuclear increase is constant either in the acute stage or in carriers, but states that if, in suspected cases, there is an increase, it is reasonable to assume that the patient has malaria or has had it. Craig,[1] on the other hand, does not believe that much importance can be attached to this finding in diagnosis.

Emerson[5] notes a relative decrease in the polymorphonuclear cells. Ross[7] finds that the polymorphonuclears, although few in number during the apyrexial periods, do not vary much from day to day. Seven days after the fever there is a marked increase, and much variation.

The lymphocytes apparently do not change much, although Talbot[13] reports a relative and constant increase in the small lymphocytes, as high as 54 per cent.

Hughes and Shrivaston,[14] working with quinine, observed that a single dose of this drug, administered intravenously, produced a leucocytosis, which they thought was due to contraction of the spleen. This leucocytosis was followed by a leucopenia, the reduction in lymphocytes being most marked, although the polymorphonuclears were also reduced. Oral administration caused a small increase in the leucocytes, affecting chiefly the large lymphocytes and monocytes. Siperstein and Litman[15] noted a rise in the white blood cell count, affecting the monocytes, and showed that when quinine was combined with alkalies there was a sharp rise in both the red and white counts, which reached a maximum on the second day.

Briefly then, the differential count in clinical malaria is characterized by a moderate leucopenia, with a relative decrease in polymorphonuclears, and a relative increase in monocytes, pronounced in the afebrile periods, and decreasing in the pyretic periods.

Perhaps the most interesting feature of the blood picture is to be found in the so-called "filament nonfilament count," or the modified Arneth polymorphonuclear count. The value of the Arneth count has been known since its inception in 1904. It is common knowledge that infection causes a "shift to the left" of the polymorphonuclears, that is, an increase in young forms. In health the number of nonfilament cells, or Arneth Class 1, ranges from 5 to 15 per cent

of the differential count. This count, done after the modified method of Farley, St. Clair, and Reisinger[17] is simple and may be quickly calculated. It is remarkably stable in health, very sensitive to infection of all types, and according to Cooke and Ponder[18] may give evidence of toxemia before the general blood picture gives any indication, and hours or even a day before the onset of clinical signs.[19] Reznikoff,[19] Minor and Ringer[16] note the finding of an unexpectedly bad picture which was apparently not justified by the nature of the case, but the reliability of which was substantiated by subsequent developments.

Cases in which the percentage of immature cells reaches 50 per cent before death are unusual. Large[21] believes a nonfilament count of 50 per cent or over carries a fatal prognosis in infections other than malaria. Kop[22] made use of this count in malaria. Blood counts were taken daily at the same hour. He noted a marked shift to the left, with the Arneth Class 1 (nonfilament) cells as high as 52 per cent, and concluded that a shift to the left in the neutrophilic blood picture was the most constant finding in malaria, that it persisted for a considerable time after the fever had subsided, and was of value in diagnosis. Also that an increasing shift to the left indicated a coming relapse. Macfie[23] reports a fatal case of malaria with a nonfilament count of 78 per cent. Henson[24] substantiated the finding of a shift to the left in infection, but stated that in malaria there was no change from the normal Arneth picture.

Nine cases of paresis treated with malaria were investigated along these lines, of which six only are reported, because these were more fully investigated. No attempt was made at selection, the cases being taken as they were admitted for treatment. All cases were inoculated by the intravenous route with benign tertian malaria from other paretics. Of the six cases, two were complicated, necessitating a termination of the malaria, which was done by oral administration of quinine. In one case the fever subsided spontaneously, and in one case the parasite was never found in the blood, although the patient had two rigors similar to those of malaria, occurring too long after inoculation to be due to protein shock.

In order to keep any personal error constant, the blood for all counts was taken by me, and all counts made by me personally. From time to time these counts were checked by other workers, and in cases of disagreement, were recounted until they checked. Two white cell counting pipettes only were used for the entire series of counts, and all total counts were made with the same dilution, using the same counting chamber for each count. The differential counts were made on carefully prepared cover slips, stained with Wright's stain, and mounted. Two hundred cells were counted in each differential count. It has been shown by Cooke and Ponder[18] that the error in the nonfilament count is so small that it is sufficient to count 50 cells. In all cases reported 100 cells were counted. These are reported, not as a percentage of the differential count, but as a percentage of 100 neutrophiles, thus making the nonfilament count correspondingly higher than that of other observers. Twenty per cent was taken as a high normal.

When possible, counts were made before inoculation to establish a normal for the individual. All counts were made at the same hour daily, in all cases. No attempt was made to follow the chills. It will thus be seen that some counts

were made during periods of fever, and some in the afebrile period. This may account for the daily variation found in all cases. The temperature of the patient at the time the count was made was noted. It was found that this bore no constant relation to the counts in any way, and hence it is not reported. The patients were followed daily as long as they remained in the hospital.

LEUCOCYTE COUNT

In all six cases there was a leucopenia, with a gradual return toward normal at the end of the infection, usually beginning soon after the termination of the fever. The leucopenia was marked, two cases falling below 2000 cells. The return to normal was gradual in all cases. The leucopenia was delayed as long as forty-eight hours after inoculation. One case showed a transient rise after inoculation, followed by a fall. Both complicated cases showed a pronounced leucocytosis at the onset of the complications.

DIFFERENTIAL COUNT

A. *Neutrophiles.*—Four cases showed a rise in the neutrophile count twenty-four hours after inoculation. The count varied from day to day, but all cases showed a progressive fall, except the complicated cases. Of these one showed a marked rise (to 88 per cent), the count remaining high, with a slow return toward normal at the end of the stay in the hospital. In both complicated cases the neutrophile count remained higher than in uncomplicated cases. There was a marked fall at the termination of the fever in three cases. One case subsided spontaneously (without quinine) and showed the same marked drop after the abatement of fever. The fall occurred two or three days after the pyrexial period, and was abrupt, continuing low, then gradually rising toward a normal level.

B. *Lymphocytes.*—Small and large lymphocytes have been grouped together in all cases.

The lymphocytes showed the same diurnal variations. They rose gradually throughout the infection. Of the two complicated cases, one continued at about the same level, and one showed a marked drop. Three cases showed a marked rise at the termination of the fever, occurring on the second or third day. A fourth subsided spontaneously (without quinine) and showed the same relative lymphocytosis.

The striking feature is the approach of the neutrophile and lymphocyte curves after the fever has been terminated. This occurred in all cases except those which were complicated. It occurred in the case which subsided spontaneously, and also in the case in which no parasites were found.

C. *Monocytes.*—There was a small increase in monocytes following inoculation, which usually occurred before the appearance of the parasite in the blood, or of rigor, and was of some value in prognosticating the appearance of chills. The maximum monocytosis found in the six cases was 12 per cent. One case showed a drop about halfway through the course, the count remaining low throughout the duration of the patient's stay. One complicated case showed a material drop at the onset of complications. The lowest count was found in the case in which inoculation was unsuccessful. Three of the cases showed a con-

siderable rise after termination of the fever by quinine. One case subsided spontaneously without quinine, and showed the same rise. Two cases were not materially affected.

D. *Other Types.*—The other types of white cells showed no changes worthy of note.

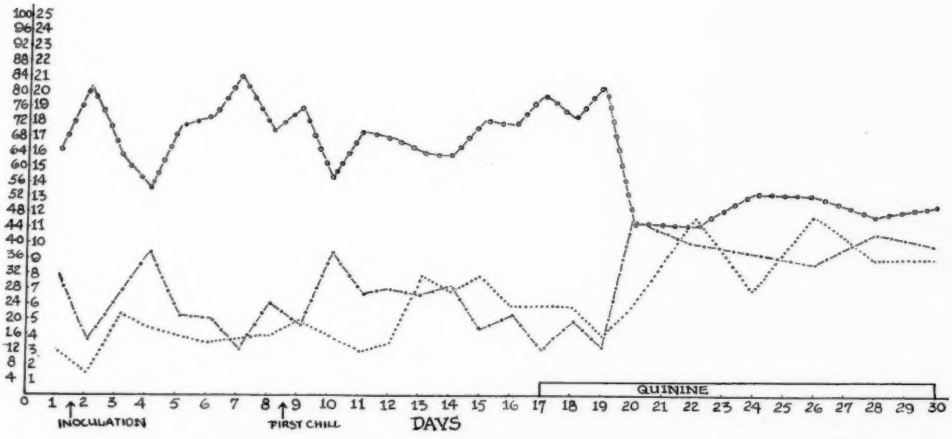

Chart 1.—Differential counts in an uneventful case. The approach of the neutrophile and lymphocyte curves is clearly shown. (Case IA. — 0 — 0 — 0 — 0 — polymorphonuclears. — . — . — . — . — . — lymphocytes. ..... monocytes.)

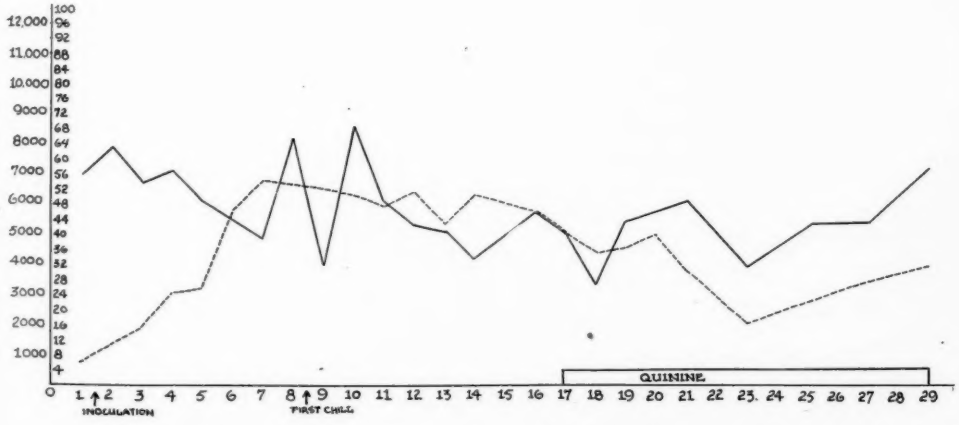

Chart 2.—A graphic representation of the total leucocyte count and the nonfilament (Arneth Class 1) cells. In this case the leucopenia was not marked. (Case IB. ———— white blood cells. — — — — nonfilament count. %, 100 neutrophiles.)

NONFILAMENT COUNT

All cases showed a rise in nonfilament cells after inoculation. This count was remarkably constant throughout. The curve was proportional to the severity of the infection. In one complicated case the count rose as high as 88 per cent before the condition of the patient appeared serious, and before the differential count showed any material change. This is the highest count ever observed at this Clinic.[21] In all cases the count remained high during the course of the fever, with a gradual return toward normal at the termination.

The drop was most marked at the termination of the febrile period. Two cases did not show this marked drop (one subsided without quinine), and the patients were discharged in good physical condition with nonfilament counts of 66 per cent and 44 per cent respectively.

In all cases where inoculation was successful, as evidenced by chills and

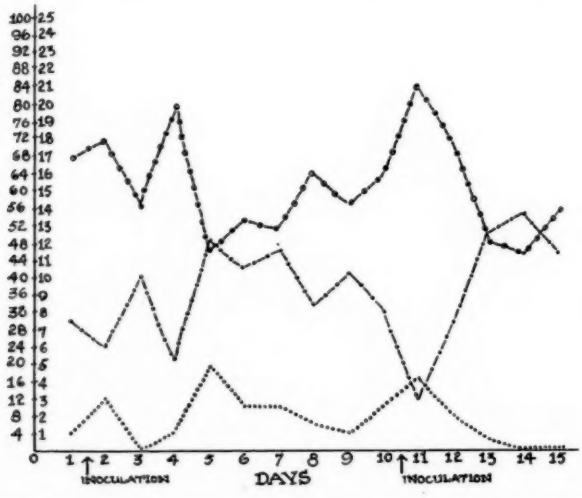

Chart 3.—Inoculation was not successful in this case. The differential count does not show the same variation. The monocytes are relatively few in number. (Case IIA. — 0 — 0 — 0 — 0 — polymorphonuclears. —.—.—.—.—. lymphocytes. ..... monocytes.)

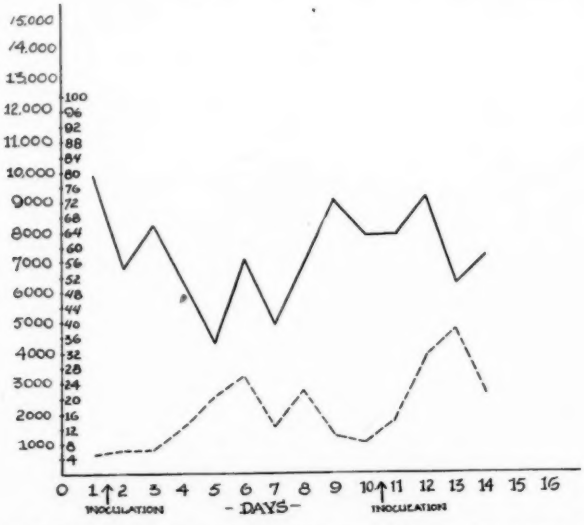

Chart 4.—Leucopenia is slight, and the nonfilament count did not rise above 38 per cent. (Case IIB. ———— white blood cells. — — — — nonfilament count. %, 100 neutrophiles.)

the appearance of the parasite in the blood, the nonfilament count reached 50 per cent and over. The cases in which inoculation was not successful showed a maximum nonfilament count of 38 per cent.

Charts are shown of three cases. In Case 1 there was an uneventful course of fever. In Case 2 inoculation was unsuccessful after two attempts. In Case

3 the patient became so severely ill that the course of the fever had to be terminated after three chills. This was foretold by the blood picture two days before the patient's condition appeared serious.

The monocytes are plotted on a scale four times that of the other cells to make them more apparent.

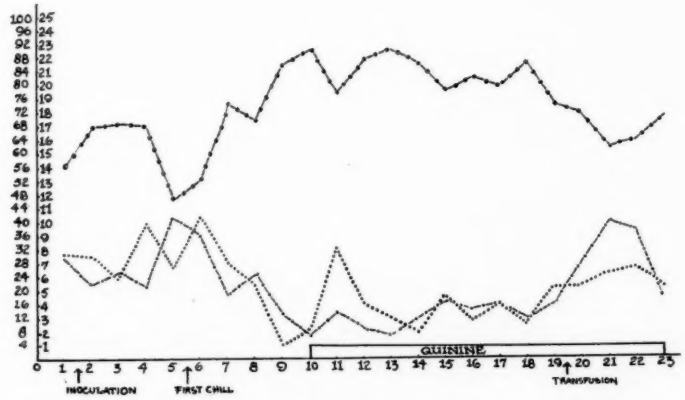

Chart 5.—This patient became so ill as to necessitate termination of his course. Note the increase in neutrophiles with a corresponding drop in lymphocytes, which was not apparent until the seventh day. Note also that these two curves never meet. (Case IIIA. — 0 — 0 — 0 — 0 — polymorphonuclears. —.—.—.—.—. lymphocytes. ..... monocytes.)

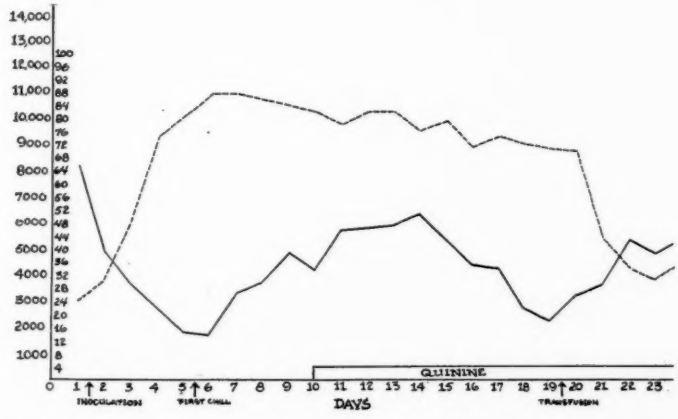

Chart 6.—The leucopenia is more marked. The nonfilament count rises abruptly to reach a maximum of 88 per cent on the sixth day, twenty-four hours before an appreciable change is noted in the differential count. Transfusion on the nineteenth day cut the nonfilament count in half, and more than doubled the white blood cells. Recovery was uneventful. (Case IIIB. ———— white blood cells. — — — — nonfilament count. %, 100 neutrophiles.)

SUMMARY

1. Daily white differential and nonfilament counts are reported in six unselected cases of inoculation malaria.

2. The blood picture in inoculation malaria differs materially from that in clinical tertian malaria.

3. Leucopenia is present throughout the course in uncomplicated cases, often marked, with a gradual rise toward normal after the infection has terminated.

4. The neutrophiles fall gradually, and show a marked fall at the termination of the infection.

5. The lymphocytes show a slight rise throughout the course, with a sharp rise at the termination.

6. The monocytes show a moderate increase, which usually occurs before the appearance of the parasite in the blood.

7. Other types of cells are not materially affected.

8. There is a marked increase in the nonfilament count, which is proportional to the severity of the infection. The count remains high throughout. It is of value in prognosis and diagnosis.

9. There is a noticeable difference in the blood picture in complicated cases.

10. Inasmuch as these changes occurred in all cases, it is doubtful whether quinine has played any part in their appearance.

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A REVIEW OF GRANULOCYTOPENIA (AGRANULOCYTOSIS)*

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THE term granulocytopenia more correctly expresses the actual condition existing in that disease known more commonly as agranulocytosis than any that has been suggested. It has the further virtue of being consistent with the accepted nomenclature describing variations in numbers and percentages of white blood cells. Any term that is expressive of the essential pathology in agranulocytosis should be adopted, and furthermore, it should possess the added virtues of simplicity and pronounceability. The term, agranulocytosis in its strict sense, means "an increased number of immature granular cells," whereas granulopenia expresses "a decrease of the granular cells." The term granulopenia fits the classification proposed by Boerner,[1] which should be adopted and which is given below.

Neutropenia—A decrease in neutrophiles
Basopenia—A decrease in basophiles
Eosinopenia—A decrease in eosinophiles
Lymphopenia—A decrease in lymphocytes
Monopenia—A decrease in monocytes
Granulopenia—A decrease in granular cells
Thrombopenia—A decrease in thrombocytes

I would suggest the addition of the last two terms in the above table as fitting the classification and expressive of the pathology in agranulocytosis.

Granulopenia seems to be a disease which may be acute or chronic, severe or mild, depending upon the extent to which the granular cells are decreased. The condition known as agranulocytosis, is probably the most extreme state of the disease in which the granular cells practically completely disappear from the peripheral blood, this, in turn, being followed by local or general sepsis, and usually by death.

A mild or chronic granulopenia undoubtedly exists in some patients, and may be responsible for the existence of certain symptoms and syndromes. Roberts and I[2] have attempted to describe a variable clinical condition which has its cause in a decreased number of granulocytes. This condition seems to be characterized by weakness, easy tiring, tendency to fatigue and chronic exhaustion, the severity of these symptoms being dependent upon the extent of decrease in the granulocytes. We were able to show, from a study of 8000 records from private practice that the typical granulopenic patients are usually women between the ages of forty and sixty. It is also this type of patient in which agranulocytosis is most often seen, and one who has a chronic granulopenia should always be regarded as a potential candidate for the development of the acute attack.

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Acute granulopenia, in which the white cell count may fall as low as 2000 with 25 per cent neutrophiles, is characterized by a somewhat sudden onset of lassitude, weakness, a tendency to sleep, and frequently the development of ulcers in the oral cavity, or in any area of the body that is normally inhabited by bacteria. These bacteria may be nonpathogenic under normal conditions, and so long as the individual is protected by the daily conferred immunity of the granulocytes, but when this is decreased, they invade the tissues, producing ulcers of various types. It is probable that the development of ulcers and other evidences of infection stimulates the bone marrow to renewed activity and thus aids in the recovery of the patient.

I will now discuss in detail the subject of agranulocytosis, which might more correctly be termed acute fulminant granulopenia.

HISTORICAL

The disease now known as agranulocytosis was first described in detail and given a name by Schultz[3] in 1922. There is little doubt, however, that it had existed for many years prior to that date, since Senator[4] described four patients with fatal pharyngitis in 1888, and Brown,[5] in 1902, described a case of "acute primary pharyngitis with extreme leucopenia," in which the white cell count was 200. Furthermore, Baldridge and Needles[6] have found a case of undoubted agranulocytosis in the records of the University of Iowa Hospital in 1910. Larson and Barron[7] in 1913 reported "a case in which the fusiform bacillus was isolated from the blood stream." Their patient died after running a septic course with ulceration of the upper jaw, with a white cell count of 2400 and fusiform bacilli in the blood stream. I feel that they were dealing with what we now regard as agranulocytosis, and their report is of considerable importance due to the association of the fusiform bacillus, an organism that must be regarded with suspicion as an etiologic agent in this disease.

An eighteen-year-old boy with recurrent agranulocytosis has recently been described by Rutledge, et al.[8] and they refer to Leale's[9] report of the same patient when he was an infant. It is probable that the disease has existed for many years before Schultz's first report and it is also probably true that the incidence has increased considerably since 1922. Whether this increase is due to an actually increased number of cases or to the fact that the attention of the medical world has been called to the condition cannot be determined, though the former view is probably correct.

Various names have been applied to the condition: as, "Angina agranulocytica" by Friedmann,[10] "mucositis necroticans agranulocytica" by Weiss,[11] "sepsis with granulocytopenia" by David,[12] "malignant neutropenia" by Schilling,[13] and a recent editorial in *The Journal of the American Medical Association*[14] refers to the condition as "granulocytopenia." The term, "agranulocytosis," is still in current use; but the term, "agranulocytic angina," should be abandoned, since many of these patients show no oral lesions. However, it may be applied to that class of patients showing oral lesions, as they usually die of the overwhelming infection, whereas the acute, fulminating type seem to die from the mere absence of neutrophiles without showing any evidence of infectious processes.

CLASSIFICATION OF GRANULOCYTOPENIC CONDITIONS

1. Agranulotoxicosis, in which the neutrophiles may entirely disappear, usually following chemical poisons, as benzene and arsenic. Weiskotten[15] has demonstrated the toxic effect of benzene on the bone marrow as regards its capability of producing neutrophiles, while Talley and Griffith[16] reported a case in a negro following arsphenamine therapy. Mme. Pouzin-Malegue[17] reports a similar instance and ascribes arsenical therapy in syphilities as being responsible for at least eight collected cases. The depressing action of arsenic on the granular elements of the bone marrow has been shown by Dodd and Wilkinson,[18] Wheelihan[19] and Farley.[20] McCord[21] brings out the depressive action of benzene in an excellent paper. I[22] have been able to produce experimental agranulocytosis in rabbits by the subcutaneous and intraperitoneal injection of small doses of benzene over long periods, in which the animals developed first a chronic granulopenia, this being followed by an acute attack with mouth ulcers, sepsis, and death. The red blood cells and platelets were unaffected, and the conclusion was evident "that the smaller the dose of benzene, the more selective is its affinity for the myeloblastic tissues." It is very likely that this class of substances is responsible for some of these cases.

2. Agranuloradiation, in which the bone marrow is temporarily depressed following excessive dosage of x-ray therapy. This happened in a patient I recently reported[23] and also involved the red cells as well as the granular cells. Clark[24] states that radiologists are always aware of this effect due to excessive x-ray dosage, for which reason extreme caution should be used in the regulation of the dosage in the treatment of patients with low white cell counts. Waters[25] makes the pertinent comment regarding the effect of x-ray on the tissues, "small doses stimulate, large doses destroy."

3. Agranulosepsis, a neutropenia due to the effect of unusual bacterial toxins or to an unusual response of the bone marrow to ordinary bacteria. Of the many bacteria isolated from the oral lesions and the blood stream of these patients, the Bacillus pyocyaneus has been found in a number of cases, and it is claimed that the introduction of this organism into laboratory animals will provoke a marked leucopenia (Lovett,[26] Linthicium,[27] Windham,[28] Keeney[29]). Roberts and I,[30] however, were unable to produce a leucopenia with a Streptococcus hemolyticus isolated from the blood stream of a typical, fulminating case. Norris[31] states that his recently isolated saccharomycete produces a marked leucopenia in laboratory animals.

It is probable that many of these patients should fall into this class; it may be possible that the large number who give a history of oral infection prior to their attack of neutropenia had a depression of the bone marrow due to invasion by mouth organisms. Such cases have been reported by Cannon,[32] Dyer and Helwig,[33] Whitehead,[34] Call, Gray, and Hodges,[35] Kastlin,[36] Thomson,[37] Moore and Weider,[38] Skiles,[39] Talley and Griffith,[40] Blumberg and Peace,[41] Kracke,[23] and others. Bacterial toxins or dead bacteria could possibly produce a neutropenia, as suggested by Bromberg and Murphy's[42] patient developing a marked neutrophilic leucopenia following typhoid prophylaxis.

4. Agranulocytosis, apparently a disease entity in which an unknown agent produces a depression of the bone marrow, this resulting in loss of neutrophilic

resistance, with subsequent overwhelming infection in those areas of the body normally inhabited by bacteria. This class should also include that type of case which is characterized by loss of neutrophiles and death without evidence of infection.

5. Aleucemic lymphatic leucemia, in which the granulocytes may almost disappear from the peripheral blood.

6. Acute infectious diseases associated with leucopenia which is often neutrophilic in type, or a relative lymphocytosis with a low total white cell count. This blood picture may be due to an actual failure of the bone marrow to produce the normal number of neutrophiles. These diseases include typhoid, typhus, measles, mumps, malaria, influenza, dengue, and sometimes syphilis and tuberculosis.

7. Roseola infantum, a neutropenia of infancy, presenting a definite clinical syndrome, but which is not necessarily fatal.

ETIOLOGY

The etiology of agranulocytosis is unknown, though much work has been done bearing on the sequence of events in the clinical course. The question was first brought up by Schultz[3] as to whether the disease is primarily an unknown infection, resulting in bone marrow depression (this in turn followed by overwhelming infection) or whether an unknown chemical agent is responsible for the bone marrow failure to produce granulocytes.

In a consideration of the possible etiologic factors, Kastlin[43] asks the pertinent questions: Is the disappearance of the neutrophiles due to:

1. an increased peripheral destruction?
2. an abnormal distribution of the cells?
3. failure of cell development?

Up to this time there is little or no evidence to indicate that increased peripheral destruction of the granulocytes is taking place. The spleen is seldom enlarged, and the red cells are little disturbed. Roberts and I[36] were unable to demonstrate a circulating agent which showed evidence of toxicity for the neutrophiles. In this test we mixed 2 c.c. of the patient's blood with an equal quantity of normal blood of the same type. Two cubic centimeters of the latter was then mixed with an equal quantity of a second normal blood, this serving as a control. Both were incubated, and total and differential white cell counts were made at two hour intervals for forty-eight hours. No differences were observed, though supravital studies (which might have given additional information) were not carried out.

The blood of patients with agranulocytosis has been injected into laboratory animals, but no disturbance of the blood picture resulted.

There is little evidence to assume that the absence of the granulocytes is due to an abnormal distribution of the cells. I have checked both venous and capillary blood on the ten cases that I have seen and the counts were essentially the same. There is little doubt but that the abnormal distribution of cells often affects the total white cell count, due to the so-called "shifting of the vascular

bed,'' as is being so well demonstrated by Garrey.[44] However, in these instances, the relative percentage of neutrophiles is little affected.

It has been well demonstrated that the failure to observe granulocytes in these patients is due to a failure of cell development, since the bone marrow at autopsy has been found consistently poor in granular cell elements, with erythroblasts present to a normal degree. Piette[45] has described these findings in detail. Moreover, studies of the bone marrow have been made during the life of the patient by Buck,[46] Zodek,[47] and Schultz and Jacobwitz.[48] They all agree that the bone marrow function is depressed.

From a summary of evidence to date, I believe it can be reasonably concluded that agranulocytosis is primarily a dysfunction of the bone marrow and, as suggested by Roberts and myself,[30] this is then followed about four days later by the disappearance of the granulocytes in the peripheral blood. Since the normal life span of the neutrophile is about four days,[15] we were able to show that the blood stream changes were manifest about seventy-two hours prior to the clinical onset. Similar observations have been made by Ehrmann and Preuss,[49] Bantz,[50] Hunter,[51] and Lauter,[52] Ashworth and Maphis,[53] and Kracke.[23] Rutledge, Hansen-Pruss, and Thayer,[8] in carefully studying a case of recurrent agranulocytosis, noted the decreased neutrophiles prior to the clinical onset during a large number of attacks.

Assuming that agranulocytosis is primarily a dysfunction of the bone marrow, the next point in consideration of the etiology is the nature of the unknown substance responsible for this depression.

As indicated before, some of the substances brought out under the heading of agranulotoxicosis may be responsible, these including arsenicals, bacterial toxins, dead bacteria, benzene and its allied and related products. This has been well discussed by Jacobsen.[54] The fact that about 75 per cent of these cases occur in women between the fourth and sixth decades, that the disease was first reported as a clinical entity in 1922, that about 90 per cent are reported from Germany and the United States, and that benzene products probably have their widest usage in those two countries, lends further support to the possible etiologic factor lying in that field.

All who have the opportunity to study these cases should carry on thorough bacteriologic and hematologic laboratory studies so as to add further information bearing on the etiology and, in particular, should obtain thorough and complete histories with especial reference to the use of unusual foods, drugs, cosmetics, clothing, shoe and hair dyes, and other substances of a rare nature that may contain bone marrow depressing substances, as benzene or its allied products.

The large number of cases giving a history of oral sepsis or tooth extractions prior to the onset should be carefully investigated as to drugs, chemicals, mouth washes, or local treatment used during that period. Shea[55] believes that oral sepsis, and Vincent's organisms in particular, is the cause of the disease. He cites the frequent occurrence of mouth lesions in the disease and the increased incidence of Vincent's angina since the war. At Emory University we are now carrying on extensive blood work on a large series of that class of patients.

INCIDENCE

The disease occurs chiefly in middle-aged women, usually in the fourth or fifth decade with a ratio of four women to one man. Hodges[56] reports three cases in males, while Hutcheson[57] reports two males in five cases. There is no age limitation, as evidenced by Jacobsen's[54] report of an infant, Bantz's[50] case in a boy of four, Dyer and Helwig's[33] case in a boy of six. Gordon[58] describes a woman of sixty-six, and Roberts and I[30] a woman of seventy-two years.

The disease is apparently not contagious or infectious, though Hart[59] describes three cases in one family. So far as I know, there is no other recorded similar instance. It has no seasonal variation but does have geographic limitations. About 80 per cent of the cases have been reported from Germany and the United States. At this time I have found only two cases from England, reported by Garrod[60] and Batten.[61] Reports from Italy are quite numerous and in 1929 Yarin[62] stated that only three cases had appeared in the Russian literature. A small boy has died with the disease in Japan and Bakker and Kuyer[63] report one from the Dutch Indies.

It seems to occur in all classes of life and occupation, having been reported in school children, housewives, business and professional women, laborers, scrubwomen, physicians, farmers, prostitutes, and ladies and gentlemen of leisure; it seems peculiarly prevalent in the last named class. For some reason the incidence of the disease is high among physicians and nurses. The literature affords many examples of this. In the eight cases of Harkins[64] was one doctor, one medical student, one nurse. In the ten cases I have seen was one doctor and two nurses. Reports of the disease in nurses and physicians have been made by Logefiel,[65] Fisher,[66] and others. It is confined largely to the white race. Talley and Griffith,[16] however, report one case in a colored female, aged thirty-six, who had been taking arsphenamine. There seems to be no occupational etiologic factor.

SYMPTOMS AND CLINICAL COURSE

There seem to be four distinct clinical types, as follows:

1. Acute, fulminant type with no infection
2. Acute, fulminant type with localized or generalized infection
3. Chronic type with acute remissions
4. Chronic type with no acute attacks.

The acute attack sometimes begins with the patient having a prodromal period of two or three days of weakness, lassitude, malaise, and perhaps sore throat. Some have no prodromal period; the attack occurs suddenly without warning. The outstanding feature is the extreme weakness and almost complete prostration. One patient stated that she had no pain, but was so weak that "she could hardly raise her head from the pillow." They usually suffer no discomfort, unless there are ulcerations of the oral cavity. Frequently, there is a marked dysphagia and varying degrees of edema of the cervical tissues. There may be ulcers on the anus or vagina, but in some cases no ulcerations are found in any locality. Jaundice is present in about 40 per cent and Hueper[67] noted gallbladder disease in three out of seven cases. Aside from the ulcera-

tions, there are usually no positive physical findings. There is an increased pulse rate, and increased temperature may be found in those who have evidence of infection. The lungs are normal except in those who die of a terminal bronchopneumonia.

The course varies. A patient may live to show evidence of blood stream and localized infection; again, death may occur within two or three days, apparently from terminal pneumonia.

The acute, fulminant cases usually die; but, if one recovers from an attack, the course then becomes chronic (with the probability of a future remission) and the blood count slowly returns to a low level for normal with the neutrophilic percentage always low. Remissions have been observed for as long as two years,[59] but the usual time is from one to three months. Because of the probability of remissions, the reporting of recovered cases should be done with considerable reservation.

As brought out before, there also exists a chronic type of neutropenia which may never show evidence of an acute attack. The patient may have a white cell count as low as 1000, with the neutrophiles almost or entirely disappeared from the peripheral blood, and the average white cell count may range around 2000 to 3000. Such a patient, who is a physician[68] has told me that on days when his white cell count is as low as 2000, he is so weak, tired, and depressed that he is unable to go to his office. On one occasion his count fell to 800 with complete absence of neutrophiles, and he had a typical attack of agranulocytosis. It is also probably true that many individuals have mild attacks of weakness, loss of activity, and depression because of a lowered number of leucocytes. I have recently had under observation a colleague who is in splendid health, but for a period of four or five days felt weak, sleepy, tired, and depressed. During this period the leucocyte count averaged 4500 with 40 per cent neutrophiles, consisting of juvenile, band, and young segmented types. At the same time there was noted a mouth ulcer, which promptly healed when the leucocyte count reached an average of 7000 to 8000.

LABORATORY FINDINGS

The findings of greatest diagnostic value are those of the blood, in which there is a marked neutropenia with later involvement of the lymphocytes and monocytes.

The white cell count may fall as low as 100 cells per c.mm. with the total absence of granulocytes. However, a count of 10,000 with complete absence of granulocytes has been observed. I have seen one patient with a white cell count of 7000, and all lymphocytes and monocytes. The red cells and hemoglobin are little affected, except in patients whose illness is prolonged.

THROMBOPENIC GRANULOCYTOPENIA

There is often a hemorrhagic diathesis. In defining true agranulocytosis in which the original diagnostic criteria of Schultz are adhered to, then those cases showing a hemorrhagic diathesis must be excluded. However, more and more reports of associated hemorrhages, due in most instances to diminished or

absent platelets, are appearing. It is becoming evident that we can draw no hard and fast line between those cases showing neutropenia only and those showing the same condition complicated by diminished platelets, purpura, bleeding, etc. It is further evidence that in some individuals only the myeloblastic tissues are affected, in some the thrombocytopenic tissues are affected, and in some the erythroblastic are affected, or that there may occur combinations and variations in severity of any or all of the three. In the last two cases I have studied the outstanding clinical factors were multiple, generalized and profuse hemorrhages. Yet the leucocyte counts in each case were below 1000. The platelets were almost absent. The red cells hovered around 2,000,000. Therefore, all of the elements of the bone marrow were affected, and yet it cannot be denied that agranulocytosis was a predominant factor.

I have recently studied a patient at the U. S. Naval Hospital, Washington, D. C., whose white cell count has been around 2000, platelets markedly diminished, purpura and bleeding, but the red cell count relatively unaffected. Such a patient does not present the picture of true agranulocytosis as originally described, nor could it be classified as aplastic anemia. Hence, I would suggest that those patients showing an involvement of the myeloblastic tissues and the thromboblastic tissues, but showing no anemia, be placed in a separate classification. For this, I would propose the name thrombopenic granulocytopenia.

The classification, proposed name and differentiation from the so-called idiopathic purpura becomes of real and practical importance, since in the latter condition, splenectomy is a rational therapeutic procedure, whereas in thrombopenic granulocytopenia it is obvious that splenectomy would be of little value in a condition whose pathology lies in the inability of the bone marrow to produce the normal number of platelets. In those cases with diminished platelets and purpura, with the white cell count bordering on real agranulocytosis, it can be reasonably concluded that the same toxin that is affecting the production of one is also affecting the production of the other. This differentiation of the two types of purpura may explain why splenectomy sometimes fails to be of therapeutic value in cases diagnosed as simple idiopathic purpura hemorrhagica.

BACTERIOLOGY

Many types of bacteria have been isolated from the ulcerated areas, these including B. pyocyaneus by Dasse,[69] Lovett,[26] Keeney,[29] and others; Streptococcus hemolyticus;[30] Vincent's organisms, staphylococci and various types of gram-negative and gram-positive bacilli.

Blood cultures have been positive in about 10 per cent of the cases, the organisms isolated including pneumococcus (Type 3),[70] B. coli communis,[71] Streptococcus hemolyticus,[30] Streptococcus viridans,[32] B. Acidi-lacti, B. lactis aerogenes,[36] and others. In one case I was able to isolate the Streptococcus hemolyticus from the blood stream, oral lesions, urine, sputum, feces, and multiple embolic abscesses.

Various organisms, including all of those named above, have been found in the heart's blood at autopsy. Piette[45] found multiple bacterial emboli throughout the kidneys and stomach wall, and they have been noted in other organs, as

well. As a result of decreased neutrophilic resistance, widespread bacterial invasion from the gastrointestinal tract is to be expected.

PATHOLOGY

In the acute fulminant cases there may be no demonstrable pathologic lesions except the characteristic changes in the marrow of the long bones. There is a red marrow which has been referred to as liquefied, evidently due to the absence of the supportive elements of the granulocytes. The granulocytes are decreased or entirely absent, even to the myelocytic forms. The erythroblasts and erythrocytes, however, are present to a normal degree, thereby accounting for the normal color. Such findings further support the theory of the bone marrow dysfunction rôle in producing the disease. No bacteria have been found in the marrow, except those distributed throughout the body, and none of those were capable of reproducing the disease in laboratory animals.

The necrotic lesions of the mouth, anus or cervix present essentially the same picture; that is, they are characterized by the absence of a surrounding inflammatory zone, since neutrophiles are not available to form it. They may vary widely in extent, ranging from a few small superficial ulcerations to extensive gangrenous processes. Edema is often present in varying degree in the cervical tissues.

The lungs frequently show subpleural hemorrhages with areas of pleural fibrinous exudate overlying small consolidated areas which contain red cells and perhaps bacteria, but no leucocytes. The digestive tract may have ulcerations throughout its entire length.

In the acute cases, complicated by generalized infection; the heart may show the changes of toxic myocarditis, and the liver show the changes incident to septic processes, including cloudy swelling, varying degrees of fatty degeneration, and occasionally small, multiple areas of necrosis. Bacterial emboli may be widespread. The spleen is enlarged, dark red, and firm; the kidneys show evidence of cloudy swelling and may present the usual changes of acute nephritis due to bacterial toxins.

Throughout the body the inflammatory changes are characterized by an outpouring of lymphocytes and plasma cells, accompanied by fluid, with the absence of the granulocytes.

The pathologic findings vary widely, being dependent upon the duration of the disease and whether or not infection has become generalized.

TREATMENT

The treatment of agranulocytosis has been generally unsuccessful. Various antiseptic agents, as alcohol, tincture of iodine, mercurochrome, iodoform, arsphenamine, silver nitrate solutions, and others, have been used on the ulcerations, but none of these seem to affect the course of the disease.

Various agents have been injected intravenously, as antistreptococcus serum, diphtheria antitoxin, nonspecific proteins, whole blood, autogenous and stock vaccines, but none is effective. Gordon[58] claims good results with the daily subcutaneous injection of nucleinic acid. Reznikoff[72] has used daily intravenous

injections of 0.5 gm. of adenine sulphate in 25 c.c. of saline and claims three cures. All three, however, received other therapy, including transfusions. He further states that the same therapy was used in six leucopenic pneumonia patients, and four of these died.

The most widely used and probably the most valuable therapeutic procedure is frequent transfusions. The addition of new blood will serve not only to supply the needed granulocytes, but some believe will stimulate the bone marrow to further production. Certainly, the use of repeated transfusions is based on more scientific facts than is any other procedure.

Fisher[66] was the first to transfuse the blood of a recovered patient in the treatment of the disease. Although the white count was down to 600 with associated purpura, the patient showed evidences of recovery immediately following the transfusion, which evidences did not follow the previous use of transfusions of ordinary blood. Chrisman and Hinton[73] have reported three cures in as many patients by the same procedure. It is indeed fortunate if recovered patients are available for this purpose.

Many believe that the use of stimulating doses of x-ray to the long bones is of value, though caution must be observed against the use of excessive dosage, in which case actual damage is done. Friedmann[74] has been the chief exponent of x-ray therapy. He reported 43 cases treated exclusively by x-ray. He states that 23 died of complicating sepsis, that five others were moribund, this leaving 15 with 13 actual recoveries. Analysis of his report shows that actually, about one out of three recovered. Waters[25] reports four recoveries in five patients treated with x-ray and other measures. I believe it to be of value if cautiously given in small stimulating doses by an expert with careful checking of the blood picture. Hueper[67] has proposed that gall bladder drainage be instituted in those cases that have a definite history of gall bladder disease, but so few give such a history that the use of the procedure has application to few cases; even in those, the wisdom of any surgical procedure should be questioned.

Any agent that is thought to be capable of stimulating the bone marrow to production of granular cells is worthy of a trial. Among these may be mentioned various nonspecific protein substances, vaccines, sera, adenine sulphate, nucleinic acid, sera of various types, milks, etc. The intravenous injection of dead typhoid bacilli may be useful. Recently marketed preparations of colloidal sulphur have been shown to increase the leucocyte count from normal to as high as 30,000 in syphilities. It is worthy of a trial. The production of sterile abscesses with turpentine has been done by Roberts and myself.

At this time the treatment is only palliative; there is nothing specific that can be used in view of the fact that the etiology is unknown. Keilty[75] states that he observed about 12 cases in a series of 5000 cases of oral sepsis, and that all recovered with appropriate local treatment to the mouth lesions. However, I doubt the correctness of the diagnosis in his series, since he stated that no differential counts were available.

One writer states that he has used massive doses of liver extract by mouth with recovery of the patient, in spite of the fact that the white cell count was below 1000 with complete absence of the neutrophiles. Since the stimulating effect of this substance on the erythroblastic function of the bone marrow is well

known, it would seem that this form of therapy is founded on rational grounds. It certainly can do little, if any, harm.

The substance sadly needed for treatment of agranulocytosis is obviously an agent that is capable of stimulating the bone marrow and that will not damage the body tissues. Such an agent has long been sought, but is not yet available. It is equally true in this disease, as in many others, that the efficiency of any treatment is inversely proportional to the number of treatments that have been used.

From a summary of present knowledge, it seems that treatment should be based on the following lines:

1. Frequent transfusions (immuno-transfusions, if possible).
2. Stimulating doses of x-ray to the long bones, with careful checking of the changes in the blood picture.
3. Colloidal sulphur subcutaneously.
4. Massive doses of liver extract by mouth.
5. Use of local antiseptics on the ulcerations.
6. Symptomatic treatment, with particular reference to cardiac stimulation.

PROGNOSIS

Approximately 250 cases have been reported with a mortality of 85 per cent. The 10 cases that I have seen are dead. Mandelbaum[76] recently reported 4 deaths in as many patients. Rosenthal[77, 78] has stressed the point that, in patients with counts below 1000, death will likely occur, and in those with counts above that figure, the chance for recovery is good. This observation was based on 5 deaths and 5 recoveries in his series of 10 patients. It is probably true that the prognosis depends, to a large degree, upon the severity of the neutropenia, though Wyatt[79] reported a white cell count of 700 with recovery, and Call, Gray and Hodges,[35] one of 640. I[80] have recently reported a patient with a white cell count of 470, followed by temporary recovery of two and one-half months duration. However, this patient died of a third attack. Therefore it is well to bear in mind the tendency of the recovered patients toward remission. If final reports on these patients were available, I believe that the mortality rate would appreciably increase.

CONCLUSIONS

1. A classification of granulocytopenic conditions, based on probable etiologic factors, is suggested.
2. A clinical classification of the disease is suggested.
3. The condition is briefly reviewed, with particular reference to possible etiologic factors.
4. Accumulated evidence points to the disease being primarily a bone marrow depression, followed in some instances by localized or generalized infection, or both.
5. It is apparently a clinical entity.
6. The name thrombopenic granulocytopenia is proposed for those cases complicated by diminished platelets and hemorrhages.
7. The treatment seems to be of little value and the prognosis remains essentially as poor as when it was first reported.

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PRESENT STATUS OF THE STUDY AND TREATMENT OF LEUCEMIA*

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LEUCEMIA is, at present, a type of disease which has received much study, but which is still an unsolved problem. Although it is a comparatively rare disease, it is of importance as at its basis is the very fundamental problem of blood production, as well as the question of the nature, therapy, and abnormal physiology of neoplastic disease. The following review summarizes a few selected points, which may be suggestive for future research.

Etiology.—The cause of leucemia is still unknown. There are many features which suggest a relationship to cancer, inasmuch as a type of body cell appears to lose its ability to mature.[1, 2] In this respect it is somewhat suggestive of the process which affects the red blood cells in pernicious anemia. Some of the neoplastic characters of the white blood cells in leucemia are the uncontrolled growth, the tendency to form secondary foci of growth (metastasis), the progress to a fatal termination with cachexia, the neoplastic type of metabolic rate of the cells,[3] the maturation with roentgen ray irradiation, the failure to transmit the disease by inoculation of human beings with the blood of affected patients, the absence of bacteriologic data of an etiologic nature, and the birth of perfectly normal children by leucemic mothers. The cells appear to have a different chromosome number from normal body cells, which may account for their altered growth potentialities. The leucemia cells appear to be physiologically different from the type of cell to which they belong. Thus, while lymphocytes are considered as being associated with immunologic processes in tuberculosis, a fulminating form of this disease may be present with lymphatic leucemia.[4] Leucemia-like blood pictures occur in lower animals,[5] and a type associated with an inoculable agent occurs in fowls.[6, 7]

Symptoms.—The first symptom of leucemia is ease of fatigue. The symptoms as a whole, fall into four groups: those associated with an increase in the basal metabolic rate (nervousness, abnormal perspiration, loss of weight); those associated with enlarged organs and glands (pressure symptoms, pains, cough, diarrhea, constipation, frequency of urination, and similar symptoms on the part of any organ the function of which is disturbed because of the abnormal growth); symptoms associated with anemia and myocardial insufficiency (dyspnea, edema, fatigue); symptoms associated with abnormal metabolism following the gradual progress of the disease (cachexia). The progressive anemia, and, in lymphatic and acute leucemias, the blood platelet deficiency, are the result of crowding out of the formative cells by the leucemic tissue. Since the present treatment is symptomatic, it follows these four lines.

Types of Leucemia.—Any of the cells of the hemopoietic system may be involved in the process—myeloid, lymphatic, monocytic, plasma cells, as well as tumor cells of various kinds. In neoplasms of some organs, very few of the cells

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enter the blood stream, whereas in others the number in the peripheral circulation is appreciable. The more advanced the stage of the disease process in the patient, the more primitive is the dominant cell in the bone marrow, lymph nodes, and blood. As the primitive cells of most of the types of leucemia are small cells with round nuclei, scanty, basophilic cytoplasm, reticular chromatin, and nucleoli, there is often some confusion in identifying their class. In acute leucemias this type of cell may predominate, and because of a superficial resemblance to small lymphocytes, they are occasionally called lymphatic leucemia, whereas the acute myeloid type is probably the more common. The change from a chronic myelogenous leucemia to the myeloblastic type at the end of the disease has often given rise to the impression that a myelogenous leucemia has turned into one of the lymphatic type. All leucemia patients, if they live long enough, end up in the "blast" stage, that is, the cells appear in less and less mature stages in the blood stream and the more immature forms are seen in greater numbers in the blood forming organs. When the blood forming cells remain confined to the bone marrow and lymphoid tissue, but few of the immature cells reach the peripheral circulation (aleucemic leucemia). When the metastatic foci, however, develop in other organs, especially those that are in constant motion, many immature forms are dislodged and the white blood cell count rises and the typical leucemic picture is seen.[8] The disease may run an aleucemic course and terminate with a high leucocyte count, the increased numbers sometimes appearing very shortly before death. With any type of leucemia, the appearance of increasing numbers of primitive cell forms (primitive "blasts") heralds a bad prognosis.

Treatment.—The therapy of leucemia, at present, is purely a treatment of symptoms. The treatment, especially of the myelogenous type, with forms of arsenic (Fowler's solution) was the method in most common use before the advent of the roentgen rays. After the general use of irradiation, arsenic fell into relative disuse, but recently attention has once more been called to its value.[9] Benzol has had a somewhat similar history, but its effects are less easily controlled and aplasia of the red bone marrow is a theoretic, if not actual, danger, at least in certain individuals.

The use of the roentgen ray or of radon has frequently been misapplied. There is occasionally a feeling that this type of radiation "kills" cells, implying a toxic or necrotizing effect. With blood cells, however, the action is of a different nature, at least with therapeutic doses. Myelocytes, metamyelocytes, young polymorphonuclear leucocytes, medium sized and small lymphocytes are stimulated to go through the rest of the stages of their normal life process, and to die of senility. This elapse of time, between the day of application of the radiation, and the time when the cells are eliminated, is often called the latent period after roentgen rays. The action on primitive myeloblasts, lymphoblasts or monocyte blasts, or the cells in the "blast" stage, is quite different. These cells are apparently stimulated to rapid division and reproduction, and the disease process is made worse rather than better. Large lymphocytes and cells of the monocyte group are but little affected by therapeutic doses of roentgen rays.

After appropriate treatment with effective doses of roentgen rays or radium, there is a progressive morphologic maturation of the red and white blood cells,

well seen in the polymorphonuclear series. On the day after the treatment there is usually an increase in the white blood cell count and the number increases until the cells reach the stage of active leucocyte movement, when they leave the blood stream by wandering out of the vessels through the mucous membranes of the mouth and probably the stomach.[10] Under similar conditions the lymphocytes are discharged through the mucous membrane lower down in the intestine.[11] The increase in maturation of the cells can be noted by the increase in number of polymorphonuclear leucocytes containing nuclei with many lobes instead of the single round nucleus of the metamyelocyte. It has been shown[12] that there is a progressive increase in the morphologic evidence of age under these conditions. It is characterized by a marked "shift to the right" in the Arneth count.[13] There is a demonstrable maturation of irradiated cells as shown by the increase in neutral red staining granules and the decrease in Janus green granules after irradiation, the former granules being characteristic of old cells, the latter, of young ones.[14]

The roentgen rays, then, stimulate certain cells in some stages to grow old and die of senility or be eliminated, while in other stages the cells are made to grow more rapidly. The first effect is desirable, the second, undesirable. Therefore, since both of these changes take place every time a patient is exposed to the radiation, it is advisable to use this type of therapy as few times as possible, especially as each treatment becomes less effective. It should be used only when (a) there are pressure symptoms from enlarged glands or spleen; (b) when there is a high white blood cell count associated with a high basal metabolic rate; (c) when the progressive invasion of the bone marrow by the leucemic tissue is crowding out the erythroblastic tissue and is resulting in a continuous fall in the red blood cell count and hemoglobin content. There is no indication for a routine irradiation at certain intervals, as the radiation does not cure the disease but has a specific effect on the immature cells in the body at that given time. Treatment may be applied over any part of the body, preferably a vascular area, such as the spleen or mediastinum. Roentgen ray or radon treatment is contraindicated when the majority of the leucocytes are in the "blast" stage, as in the acute leucemias, or the myeloblastic, lymphoblastic or monocytoblastic stage of the chronic leucemias. This is the so-called refractory type or stage of the disease.[15, 16]

Blood transfusion has some effect in alleviating some of the symptoms, especially when there is a marked degree of anemia. Frequently there is a temporary decrease in the white blood cell count after a transfusion. Iron, in simple forms, is of some value in chronic lymphatic leucemia, and whole liver is also of some use. Liver or liver extract appears to be of little value in the chronic myelogenous form.

Lugol's solution has a definite effect, especially in chronic lymphatic leucemia, in relieving some of the symptoms associated with a high basal metabolic rate.[17]

Because of the low resistance to infection and tendency to bleed in leucemia, special preparations must be made before any operative procedures are carried out. It is advisable to give roentgen ray treatment until the white blood cell count is nearly normal, as the leucocytes prevent the formation of an efficient

clot in myelogenous leucemia and the platelets are deficient in number in the myelophthisic anemia in the lymphatic and acute types of the disease. Routine removal of the teeth as foci of infection is contraindicated. Tonsillectomy has been of no great value in this disease and splenectomy has no marked effect in prolonging the life of the patient or stopping the disease.

At present all that can be hoped for from the treatments in use is the temporary restoration of the patient to a degree of efficiency which will enable him to live a useful life at least during part of his disease. The average life of the patient is about three and a half years, with fairly wide extremes which appear to be related to the constitution of the patient and not to the therapy.[18-22]

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TREATMENT OF THE ANEMIAS*

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THE most essential requirement in the treatment of an anemia is the accurate recognition of the particular variety of the condition which is present. This is absolutely necessary because certain therapeutic measures which are very efficient in the treatment of some anemias are entirely useless in others. For example, liver extract and ventriculin are highly potent in pernicious anemia but are ineffective in the chronic secondary anemias. On the other hand, large doses of iron produce splendid results in some varieties of the latter condition, but they are without effect in pernicious anemia. Splenectomy is a specific cure in chronic hemolytic jaundice but is contraindicated in the myelophthisic anemia which is associated with Hodgkin's disease, and which is improved by the therapeutic application of the roentgen rays. The anemia of myxedema yields readily to treatment with desiccated thyroid gland but is uninfluenced by liver or desiccated stomach which is sometimes given because the patient is thought to have pernicious anemia.

THE TREATMENT OF CHRONIC SECONDARY ANEMIA

The term secondary anemia was originally introduced to include those anemias which were due to recognizable causes, in contrast to the primary anemias of unknown etiology. For some years, however, it has been customary to include in this group also those anemias with a low color index, despite the fact that their cause was unknown. This appears to be a logical grouping and, therefore, the anemias which will be included under the head of "chronic secondary anemia" in this article are those due to known causes or those which have a low color index, or those meeting both requirements.

It is difficult to estimate accurately the frequency with which the various anemias are encountered in the practice of medicine but undoubtedly chronic secondary anemia has the greatest incidence. This is true because there are so many different causes responsible for the condition. It may be associated with chronic blood loss due to uterine disorders, hemorrhoids, bleeding peptic ulcer, neoplasms, improper diet, infections, and a large number of chronic long continued diseases.

The essential requirement in the treatment of secondary anemias is to remove or control the underlying cause if this is possible. Until this is accomplished, it is too much to expect that various measures employed to overcome the anemia will produce their full effect. The first step, therefore, in treatment consists in subjecting each patient to a thorough study in an effort to discover the cause of the anemia and removing it, if possible. If the anemia is secondary to

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bleeding, every effort should be made to check this. Various infections, if present, should be eliminated but caution should be used in ascribing the anemia to various foci, such as teeth and tonsils. There should be reasonable assurance that they play a significant causative rôle in the production of the anemia before they are removed. Extraction of all the teeth has not only failed to cure the anemia in many instances, but has actually done harm on account of the transient resultant mouth infection and the subsequent inability to ingest a normal diet for a considerable period of time. In the anemias associated with Hodgkin's disease and the leucemias, improvement can only result from irradiation with radium or the roentgen rays.

In many types of secondary anemia it is beneficial to administer *large* doses of iron and in at least one type it has a specific action. This variety is the idiopathic microcytic or hypochromic anemia which appears to be increasing in incidence during the past few years. It occurs in women of middle age and is characterized by asthenia, vague gastrointestinal complaints, decreased resistance to infection, and a favorable response to iron medication. This condition has not received the emphasis to which it is entitled, as it occurs rather frequently. To overlook the condition and omit the proper treatment is regrettable for the response to iron is very striking. It resembles chlorosis in some respects, but differs in the age incidence, as it occurs most frequently in women between the ages of thirty-five and forty-five years. The etiology is unknown although probably the cause is concerned with the metabolism of iron, and one view regards it as an iron starvation resulting from defective absorption.

The important consideration when administering iron is not the nature of the preparation, but the size of the dose. As Witts[1] very aptly states, "Few facts in medicine are so unanimously believed by the expert, so strongly backed by evidence, and so little known to the profession, as the absolute necessity of very large doses of iron in the treatment of these chronic anemias." Poor results in the past have been due in many instances to an inadequate dosage. A convenient method of administering iron is in the form of ferric ammonium citrate which may be given in a 50 per cent solution in doses amounting to 4 to 6 grams (60 to 90 grains) daily, but probably the simplest and most satisfactory form is reduced iron given in 0.5 gram (7½ grain) capsules three times daily. All of these dosages are very much larger than are advised in most textbooks or in the pharmacopeia, but satisfactory results will not be obtained unless these amounts are used. Contrary to the usual belief, these large doses are well tolerated and rarely cause digestive disturbances.

The dietary habits should be investigated in all patients with a secondary anemia when the cause is not obvious, for there is considerable evidence that the ingestion of an obviously abnormal diet over a long period of time may cause a definite anemia. This occurs principally in three types of patients, (1) those who have difficulty in consuming food on account of cardiospasm or other mechanical causes, (2) those who consume a most abnormal diet as the result of personal whims, and (3) patients with hypertension or nephritis who have existed on a diet, usually exceedingly low in protein, for a long period. The cure of the anemia can only follow the taking of a proper diet which should be prescribed for the patient.

THE TREATMENT OF PERNICIOUS ANEMIA

Before the year 1926 the treatment of pernicious anemia was highly unsatisfactory as there was no form of therapy, with the possible exception of blood transfusions, which would prolong the life of a patient with this disease. With the introduction of liver as a form of therapy by Minot and Murphy[2] in the year just mentioned, liver extract in 1927 by Minot and Cohn et al.,[3] and ventriculin by Sturgis and Isaacs in 1929,[4] the entire outlook concerning the disease has been altered. The experience of a fairly large number of observers has demonstrated that the anemia of pernicious anemia can be made to disappear following the proper use of any one of the three forms of treatment in uncomplicated cases. The essential part of the treatment is to administer an adequate amount of liver, either cooked or uncooked, a potent liver extract or ventriculin (desiccated, defatted hog stomach) until the blood returns to normal. When this has been attained, a sufficient quantity of one of these substances must be given regularly in order to maintain the blood within normal limits. The average initial dosage of any one of these three forms of treatment which will cause the red blood cell count to rise to normal at the rate of about 400,000 red blood cells per cubic millimeter per week, is as follows: Liver, ½ pound daily; liver extract, 5 to 6 vials daily (each vial representing 100 grams of liver), or ventriculin, 10 grams daily for each million deficit in the total red blood cell count. The liver extract may be given either dissolved or suspended in water, tomato juice, ginger ale, or grape juice.

The effect of the treatment is prompt and striking. Within three to six days the patient shows evidence of improvement as indicated by an increase in appetite, a disappearance of gastric symptoms, such as nausea and vomiting, a gain in strength, and a fall in the pulse rate and body temperature to normal. The improvement is progressive and often after a few days to a week, a patient who has been confined to bed on account of weakness is able to sit up or walk about. Patients with uncomplicated pernicious anemia usually improve very rapidly and are able to resume a normal life within six to eight weeks after the beginning of the treatment.

The earliest change in the blood is not an increase in the number of red blood cells but an increase in the number of reticulocytes, or immature red blood cells. If the changes in cells are charted in the form of a curve, a definite rise in their number will be observed to begin between the third and sixth day of treatment. Following this there is a rapid increase until a peak of the curve is reached between the seventh and ninth day. There is then a gradual decline to about 1 per cent on approximately the fifteenth day of treatment. The height of the peak of the curve has an inverse relationship to the initial level of the red blood cell count just prior to treatment. For example, if the red blood cell count is one million, the height of the peak will be about 34 per cent; if the red blood cell count is two million, the height will be about 14 per cent; if the red count is three million or over, there will be slight if any increase in the number of reticulocytes following treatment. The red blood cells may not change during the first two weeks of treatment, or there may even be a decrease in their numbers. During subsequent weeks of treatment, however, the red blood cell count

may rise a million per cubic millimeter per week or more, so that the average increase, from the beginning of treatment until the blood is normal, is about 400,000 per cubic centimeter per week.

In some instances it has been reported that liver extract or ventriculin has failed to produce satisfactory results when used in the treatment of pernicious anemia. Careful investigation has usually disclosed one of the following reasons for this conclusion:

1. The diagnosis of pernicious anemia has been incorrect. The patient may have been suffering from one of the forms of chronic secondary anemia, aleucemic leucemia, myxedema or some other type of anemia. In my experience, liver or stomach therapy is effective only in the treatment of the Addisonian type of pernicious anemia, the anemia secondary to Dibothryocephalus latus infestation, the so-called "pernicious anemia of pregnancy" and some types of anemia associated with sprue.

2. An acute infection has been present which inhibits to some extent the efficiency of liver or stomach therapy. This may be any type of infection, such as a cystitis, pyelitis, erysipelas, pneumonia, acute tonsillitis, or any variety of infection which is associated with a febrile reaction. In all patients who have such a complication the dosage of the preparation used should be increased 100 per cent as long as the infection is active.

3. The liver extract or preparation of stomach has been of weak potency or completely inert. The only certain way to avert this possibility is to employ material which has been clinically tested and certified as having full potency. In very rare instances a patient is observed whose red blood cell count may reach a level of 2.5 to 3 million per cubic millimeter where it remains, for some unknown reason, despite large doses of liver or stomach by mouth. These patients may be treated with good results by intramuscular or intravenous injections of liver extracts which have been specially prepared for this purpose. Some such preparations when given intravenously may be followed by profound reactions, as evidenced by chills, fever, and hypertension, but undoubtedly this method of therapy is one of great promise if the cause of the severe reactions is eliminated.

In order to obtain satisfactory results, all patients with pernicious anemia should remain under observation until their red blood cell count reaches normal, although after their red count is three million per cubic millimeter or more, they need be seen at less frequent intervals. During this period of observation the patient should have the red cells counted at frequent intervals, as the level of the red count is the best single criterion of the effectiveness of the treatment. If this remains low after the patient has received the average dosage of the preparation for a reasonable length of time, the amount should be increased. The patient should be warned that a relapse will follow in six weeks to several months after therapy is discontinued.

Experience has shown that only liver or ventriculin is necessary in order to bring the blood of patients with pernicious anemia to normal. Such additional forms of therapy as iron, arsenic, and dilute hydrochloric acid appear to be entirely unnecessary. Some patients with pernicious anemia when first seen are extremely ill and have a profound anemia. All such patients should have their blood grouped for transfusion and arrangements made to have a suitable

donor at hand. Transfusions may be advisable in order to support the patient until the therapeutic effect of liver or stomach is apparent.

Although this article is primarily concerned with the treatment of the anemia of pernicious anemia, it seems proper to discuss briefly the effect of the treatment on the spinal cord complications which are not uncommon. The results attained in the treatment of this condition are far less satisfactory than those directed toward the relief of the anemia. Usually the minor neurologic manifestations, such as numbness and tingling of the extremities, completely disappear or become much less as the blood condition improves. Other symptoms, as a spastic paraplegia or loss of the sense of position of the extremities, which indicate a more serious lesion of the spinal cord, may improve but more frequently are arrested and occasionally advance despite all therapeutic efforts.

TRANSFUSIONS

Blood transfusion, either of citrated or noncitrated blood, is useful in the treatment of various types of anemia and should be considered under the following circumstances:

1. As an emergency measure to sustain life until bleeding can be checked or other effective measures introduced to combat the anemia. A patient at the point of death from traumatic hemorrhage may survive only as the result of a transfusion while appropriate surgical procedures are performed. As previously mentioned, patients who are critically ill with pernicious anemia may be temporarily improved by one or more transfusions, and thereby survive until the permanent beneficial effects of liver or stomach become apparent.

2. As a method of treating the excessive bleeding in hemophilia and thrombocytopenic purpura. In both conditions there may be a severe associated anemia and in both, the bleeding is controlled by blood transfusions. Patients with hemophilia can be carried through major operations by repeated blood transfusions, given before and after the operation. Even such minor procedures as the extraction of a tooth in such patients should be preceded by blood transfusions and the operation not performed until the coagulation time reaches normal.

3. Patients with an anemia which is curable by surgical means should not be operated upon until repeated transfusions have caused the blood to approach normal limits. Splenectomy in chronic hemolytic jaundice, Banti's disease, and chronic idiopathic thrombocytopenic purpura should not be performed without one or more transfusions preceding the operation, if an anemia is present.

4. Some believe that one or more transfusions should be advised in the anemia of chronic hemorrhage after the bleeding has been checked, in order to expedite the return of the blood to normal. While this may be indicated occasionally, it should not be done routinely, as the regeneration of blood is usually at a satisfactory rate when assisted only by a nutritious diet and large doses of iron.

SPLENECTOMY

This condition has been advised as a therapeutic measure in the anemia of pernicious anemia, the anemia associated with the leucemias, chronic hemolytic jaundice, splenic anemia, and chronic idiopathic thrombocytopenic purpura

(purpura hemorrhagica). Observations have shown that the operation is no longer indicated in pernicious anemia and the leucemias, as evidence indicates that neither of these conditions is benefited by the operation. Furthermore, the use of stomach or liver therapy produces excellent results in pernicious anemia and the roentgen ray is the most effective treatment available at present for the treatment of the leucemias.

Chronic hemolytic jaundice is apparently curable by splenectomy which is indicated if the patient's symptoms are of such intensity as to warrant a major operation for their relief. Within a short time after the operation, the jaundice disappears, the blood returns to normal, and all symptoms subside. In many patients with this disease the symptoms are very mild and a major operation is not justifiable. A conclusion concerning the advisability of splenectomy must be decided in each individual case after a careful consideration of the patient's history, physical examination, and blood studies.

Splenectomy is indicated in splenic anemia (Banti's disease), especially during the preascitic stage when cirrhotic changes have not occurred in the liver, as this is the only form of therapy which is effective. Some consider that is should be performed even when there is definite evidence of an associated cirrhosis of the liver. Before such radical treatment is carried out, however, there should be every assurance that the diagnosis is correct. The syndrome may be closely simulated by Hodgkin's disease, with an enlarged spleen and an absence of peripheral lymph gland enlargement and by syphilis with splenomegaly. Preceding the operation, all such patients should be given a several weeks' course with large doses of potassium iodide as a therapeutic test for syphilis. If no change occurs, several applications of the roentgen ray should be given over the spleen. If the splenomegaly is due to Hodgkin's disease, there should be a prompt decrease in the size of that organ.

Splenectomy must be considered in all patients with chronic primary purpura hemorrhagica (chronic idiopathic thrombocytopenic purpura), provided the symptoms are severe enough, and if they are not controlled by repeated transfusions. The results in the chronic cases are satisfactory but the operation is contraindicated in the acute type on account of the high mortality.

MISCELLANEOUS FORMS OF THERAPY

Various preparations of arsenic have been recommended in the treatment of several types of blood diseases. For many years it was used in the form of Fowler's solution in the treatment of pernicious anemia, and good results were reported. Although it may cause an improvement in the patient's condition, the beneficial effects cannot be compared with those obtained by stomach or liver therapy. Nor is there convincing evidence that arsenic alone is of value in the treatment of the secondary anemias, or that a combination of arsenic and iron is any more effective than iron alone.

Spleen marrow has been advocated as an effective therapeutic agent in the secondary anemias. It has proved ineffective in my experience, and the reported results are not as satisfactory as those observed following efficient iron therapy.

Copper has been employed to some extent in the past two years in the treatment of the secondary anemias, but the clinical results, at least in adults,

do not indicate that it has a beneficial effect. This form of therapy was introduced as a result of convincing experimental evidence that minute amounts of copper cured a nutritional type of anemia in rats.

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II. STUDIES ON PATIENTS WITH PERNICIOUS ANEMIA TREATED WITH MASSIVE DOSES OF LIVER EXTRACT

Effects on Reticulocytes, Red Cells, Hemoglobin and White Cells*

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THE clinical and hematologic effects of the administration of massive doses of liver extract have been reported on by Riddle and Sturgis,[1] and more recently by Connery and Goldwater.[2] Two of the four cases included in the series of the latter observers received transfusions in addition to the massive doses of liver extract.

It is the purpose of this paper to present data on a series of fifteen cases of pernicious anemia treated with single massive doses of liver extract, with or without transfusion, or with repeated massive doses, with or without transfusion. These data are considered under the following headings: (a) effects on reticulocytes, (b) effects on red cells and hemoglobin, (c) effects on the white cells, (d) effects on the blood sugar, and (e) effects on the blood pressure.

MATERIALS AND METHODS

Of the fifteen cases of pernicious anemia included in this study, ten were males and five were females. Eight were admitted to the hospital in their first relapse, four in their second relapse, and three in their third relapse. Five of the patients gave a history of having previously taken some form of liver therapy, whereas no history of previous liver therapy was obtained from the other ten. Six patients were admitted with red cell counts below one million, six more had counts between one and two million, two had counts between two and three million, and one had a count greater than three million. Four of the patients exhibited signs of well established subacute combined sclerosis. The other eleven showed only slight neurologic involvement. In eight of the patients, one or more of the following complications were present on admission: acute or chronic

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bronchitis, subacute cholecystitis, subacute or chronic sinusitis, chronic cystitis associated with hypertrophy of the prostate and residual urine, sloughing varicose ulcers with cellulitis, dental apical abscesses. In six of the patients the severity of the illness was of a mild degree, in three the severity was of a moderate degree, while the remaining six were critically ill. All of the cases included in this study were patients on the wards of the Third (New York University) Medical Division of Bellevue Hospital.

All apparatus used was standardized. Determinations on each patient were made by the same observers, using the same apparatus throughout the period of study. Where the condition of the patient permitted a control period of sufficient length was run to establish the range of the reticulocytes. In those cases in which it seemed that the life of the patient might be jeopardized if immediate treatment were not given, no control period was run. This fact, however, does not invalidate the results in these cases, since the time of beginning reticulocyte response, and the character of the reticulocyte curves were entirely comparable to those seen in the patients in whom there had been a control period. Daily reticulocyte determinations were made during the period of increased peripheral reticulocyte activity. Red cell counts, hemoglobin determinations, and white cell counts were made bi- or tri-weekly, and in some instances even more frequently. The "hemogram" was studied at least bi-weekly, and in many cases as often as five times weekly, usually during the period of increased peripheral reticulocyte activity. The classification of Schilling[3] was used, with a slight modification in the nomenclature. Blood pressure readings were made by the auscultatory method, using a mercury manometer. Readings were made at sufficiently short intervals so that any significant trend in the curves would be observed.

In estimating the blood sugar content, the method of Folin and Wu was used. Samples of blood were collected under standardized conditions at half hour intervals over a period of two hours. Transfusion was used only in those cases in which it seemed that the life of the patient would be endangered unless this measure was employed. While valuable information may have been adduced by the incidental use of transfusion, this procedure was not included as one of the objects of the study. The Lindemann method was used, and the amounts given were 300 c.c. to 650 c.c. per transfusion.

The liver extract* used was a commercial preparation of fraction G of Cohn, Minot et al.[4] One vial of the extract represents the material obtained from 100 grams of whole mammalian liver. The amount of a single dose varied from thirty to fifty vials. The extract was dissolved in from 200 c.c. to 300 c.c. of tap water, milk or buttermilk, and was administered as follows: Nine cases (Cases 1, 2, 3, 4, 6, 8, 9, 11, 15) received the extract into the stomach by Rehfuss tube. Vomiting[1, 2] occurred in Cases 1, 2, 4, 6, 8, 11, and 15. Four cases (Cases 7, 10, 11, 14) received the extract through a gastric lavage tube. Vomiting occurred in Case 10 only. Cases 5, 12, and 13 drank the extract in iced buttermilk. Vomiting occurred only in Case 5. It may be stated here that there seemed to be no relationship between the size of the dose, the mode of administration, the time of vomiting, the amount vomited, and the subsequent progress of the patient.

*Prepared by the Lederle Laboratories.

RESULTS

Reticulocytes.—Nine cases (Cases 1 to 9, Table I) were treated with single massive doses of liver extract without transfusion. Inasmuch as reticulocyte determinations were made at intervals of twenty-four hours, a beginning response noted as occurring on the second day actually took place between twenty-four and forty-eight hours, a response on the third day actually took place between forty-eight and seventy-two hours. Likewise, a peak noted as occurring on the fourth day actually took place between seventy-two and ninety-six hours, and so on.

TABLE I

CASE	DOSE	NO. OF DAYS TO BEGINNING RESPONSE	NO. OF DAYS TO PEAK	HEIGHT OF PEAK	NO. OF DAYS OF I. P. R. A.	ESTIMATED RETICULOCYTE CONCENTRATION	CALCULATED RETICULOCYTE CONCENTRATION
1	50	*	*	*	*	*	*
2	50	3	7	26.0%	29	0.40	0.49
3	30	3	6	32.1%	9	0.37	0.57
4	50	3	6	12.5%	**		
5	30	2	6	24.8%	21	0.43	0.47
6	50	***	***	***	***	***	***
7	50	3	4	32.0%	13	0.37	0.57
8	50	4	6	12.0%	10	0.20	0.43
9	48	2	6	22.5%	11	0.52	0.37

*No response. Red blood cells at 3 million level. Previous reticulocyte peak.
**Left hospital at own request while reticulocytes were on the increase.
***No response. Red blood cells 3.5 million.
I. P. R. A. = Increased Peripheral Reticulocyte Activity.

Formula for Estimated Reticulocyte Concentration in millions = $E\,p^r = \frac{E\,o^r}{1-r}$

Formula for Calculated Reticulocyte Concentration in millions = $E\,r = 0.73 - 0.2\,E_o$

$E\,p^r$ = Observed concentration of reticulocytes at peak of rise.

$\frac{E\,o^r}{1-r}$ = Estimated concentration of reticulocytes at peak of rise.

E_r = Calculated concentration of reticulocytes at peak of rise.

E_o = Original level of erythrocytes.

E_p = Erythrocytes concentration at height of reticulocyte peak.

r = Reticulocytes in per cent.

There seemed to be no relationship between the size of the dose, and the number of days to beginning reticulocyte response, the number of days to reticulocyte peak, the height of the reticulocyte peak, or the number of days of increased peripheral reticulocyte activity.

No reticulocyte response was noted in Cases 1 and 6. Failure to induce increased reticulocyte activity by this form of treatment in Case 1 could be explained by two facts: (a) the level of the red cells at the time the massive dose was given was above three million, (b) the reticulocytes were increased above the normal at the time of admission (9 per cent) and rose to 17.4 per cent before

treatment was given (see Chart 1, Curve 1). In Case 6 the level of the red cells at the time treatment was given was 3.6 millions[5] (Chart 1, Curve 6).

The administration of 50 vials of liver extract to Cases 4 and 8 induced reticulocyte peaks of no great magnitude, a fact which warrants explanation. In the former, Case 4, the presence of subacute cholecystitis quite likely depressed the magnitude of the reticulocyte response, although it must be pointed out that the patient left the hospital at his own request at a time when the reticulocytes were on the increase. In the latter, Case 8, the presence of chronic bronchitis and several abscessed teeth may have been responsible for the low level reached by the reticulocytes at their peak.[5] Three of the cases (Cases 2, 5, and 9) showed a satisfactory agreement between the estimated and calculated reticulocyte concentrations.[6] In three cases (Cases 3, 7, and 8) this agreement was not present.

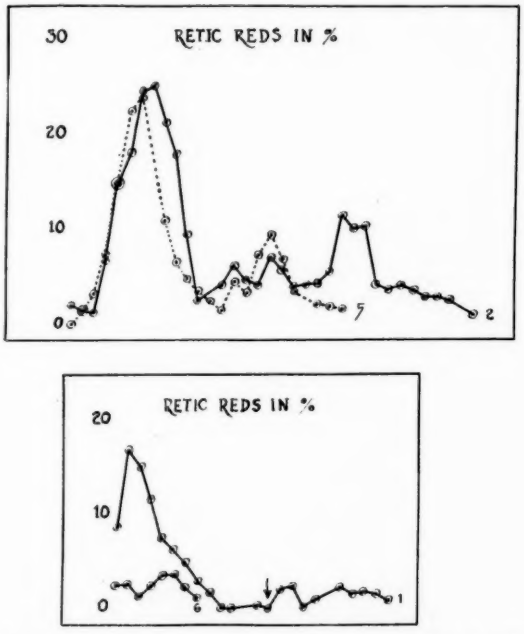

Chart 1.—Treatment given on 0 day in all cases except Case 1, where day of treatment is indicated by arrow. Curve numbers coincide with case numbers. Each division on abscissa represents one day.

Reticulocyte concentrations could not be calculated in Cases 1, 4, and 6 due to incompleteness of data. In Case 7 the lack of agreement between the estimated and calculated reticulocyte concentrations was probably due to the presence of a purulent sinusitis and numerous apical abscesses. The factors responsible for depressing the magnitude of the reticulocyte response in Case 8 may also serve to explain the lack of agreement between calculated and estimated reticulocyte concentrations in this case.

Secondary reticulocyte peaks have been observed quite frequently in patients who have been treated with suboptimal amounts of liver or a potent extract in daily doses and later with optimal amounts. The same phenomenon may be seen when the administration of a weak liver preparation is followed by the use of optimal amounts of a potent preparation.[6] Two of the cases (Cases 2 and 5),

treated with single massive doses of liver extract, showed secondary peaks in their reticulocyte curves (Chart 1, Curves 2 and 5). The conditions mentioned above did not prevail in these cases, so that the significance of the secondary peaks remains a matter for speculation. These two cases were at the extremes of the scale of dosage, receiving 50 vials and 30 vials respectively, and of the entire series ran the most favorable courses, both hematologically and clinically.

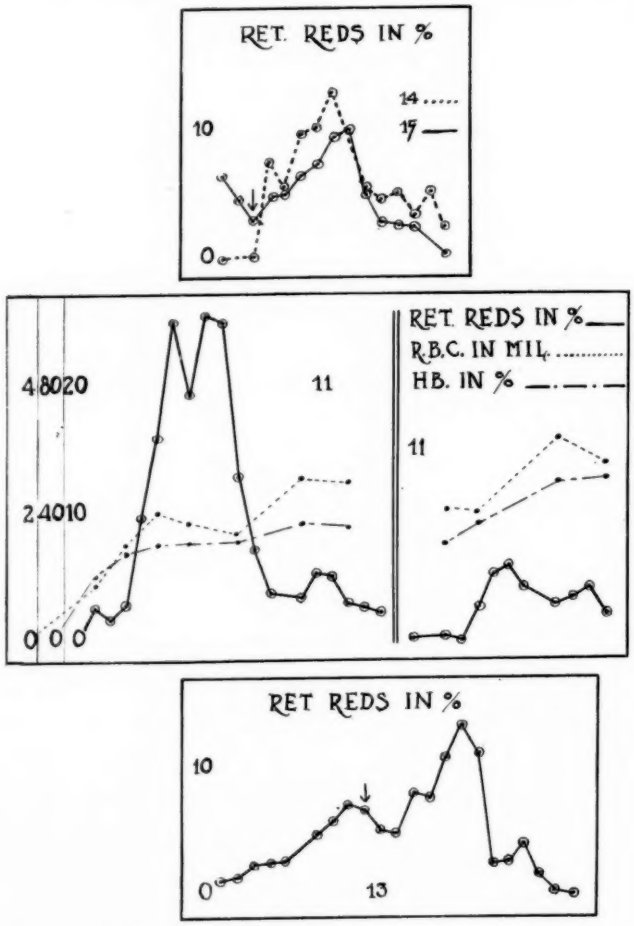

Chart 2.—Treatment given on 0 day except where indicated by arrows in Curves 13 and 15. In the latter the first dose was given on 0 day and the second as indicated. Curve numbers coincide with case numbers. Each division on abscissa represents one day. In Case 11, the double vertical bar indicates an interval of twenty-one days, at the end of which time a second massive dose was given. In Curve 13, time from 0 to arrow represents period on kidney extract.

Case 13 (Chart 2, Curve 13) had a previous period of hospitalization, during which he was treated with Marine Liver Extract* in daily doses of 90 c.c.[7] A satisfactory remission had been induced, but after the patient left the hospital he at first took the extract irregularly and eventually ceased taking it altogether. About a year later he was readmitted to the hospital in a well established relapse. During the first ten days he was given a daily dose of a kidney extract derived from 800 grams of whole mammalian kidney.[8] The technic used in preparing

*Prepared by the White Laboratories of Newark, New Jersey.

this extract was that used in the manufacture of a commercial extract of fraction G.[4] Chart 2, Curve 13 shows that the administration of the kidney extract induced a slight reticulocyte response. After ten days of this treatment, when it became apparent that little or no further increase in the reticulocytes could be expected, and that only slight clinical improvement had occurred, it was deemed advisable to discontinue this form of treatment. Accordingly, a single massive dose of 50 vials of liver extract was given. On the sixth day following, the reticulocytes reached a peak of 12.8 per cent. That the reticulocytes did not reach a higher level can probably be explained by one or both of the following facts: (a) a slight reticulocyte response had just been elicited by what were either suboptimal amounts of a potent extract or maximal amounts of a weak extract; (b) lobar pneumonia supervened while the reticulocytes were still on the increase.[5]

TABLE II

HEMOGLOBIN DETERMINATIONS BY KLETT NEWCOMER METHOD, 100% = 17 GRAMS HEMOGLOBIN PER 100 C.C. BLOOD

IN SOME INSTANCES THE HEMOGLOBIN REACHED A HIGHER LEVEL THAN THAT OF THE DAY WHEN THE RED BLOOD CELLS WERE AT THEIR MAXIMUM

CASE	EXTRACT		TRANSFUSION		INITIAL LEVEL		LEVEL FOLLOWING TRANSFUSION		MAXIMUM		TOTAL INCREASE*		NO. OF DAYS TO MAXIMUM
	DOSE IN VIALS	DATE	AMOUNT	DATE	R.B.C. IN MIL.	HB GR.	R.B.C. IN MIL.	HB GR.	R.B.C. IN MIL.	HB GR.	R.B.C. IN MIL.	HB GR.	
10	50	1/27/31	500 c.c.	1/27/31	0.53	2.1	1.25	5.0	2.6	8.6	1.35	3.6	21
11	50	10/14/30	500 c.c.	10/14/30	0.99	3.6	1.56	4.9	2.78	8.0	1.22	3.1	23
12	30	4/18/31	500 c.c.	4/18/31	0.70	3.2	1.40	6.5	2.65	7.4	1.2	0.9	10***
13	50	4/16/31	500 c.c.	4/27/31**									
14	42	8/ 1/30	650 c.c.	8/ 2/30									
	42	8/ 2/30	500 c.c.	8/ 5/30	0.65	1.6	2.4	6.0	4.8	11.8	2.4	5.8	51

*Counting from after transfusion.
**Transfused because of pneumonia.
***Erysipelas developed.

Transfusion in conjunction with single or repeated massive doses of liver extract was used in a series of five cases (Cases 10 to 14, Table II). Of these, two cases (Cases 10 and 12), received a single transfusion of 500 c.c. of whole blood and a single massive dose of 50 vials and 30 vials of liver extract respectively. The response of the reticulocytes in these two cases was quite similar to that seen in comparable cases treated with single massive doses without transfusion.[9, 10] The reticulocyte curves of these two cases were almost exact duplicates of the first part of Curve 11, Chart 2, q. v. which represents the curve of the reticulocytes following a single massive dose, plus transfusion. This case is treated separately because a second massive dose was given later, as will be discussed below. The similarity of this curve to Curves 2 and 5, Chart 1, those of cases treated with single massive doses without transfusion, is quite striking.

The first part of Curve 11, Chart 2, already mentioned, shows the reticulo-

cyte response following a single massive dose and a transfusion, in Case 11. The second part of Curve 11, Chart 2 shows the reticulocyte response following a second massive dose, which was given when it had become apparent that no further increase in red cells or hemoglobin could be expected following the first massive dose of liver extract. Although the size of the second dose was the same as that of the original dose, and the level of the red cells was practically no higher than it was at the time the first dose was given, the reticulocyte response following the second dose was of a much smaller magnitude than that of the original reticulocyte response. The explanation for this probably lies in the fact that a major period of increased peripheral reticulocyte activity had just been completed.[6]

Case 14 received two doses of 42 vials each and transfusions of 650 c.c. and 500 c.c. respectively, all within a period of four days. The response of the reticulocytes to this treatment is shown on Chart 2, Curve 14. Two features are worthy of comment. First, the beginning reticulocyte response did not occur any sooner than that in cases receiving smaller doses. Second, the magnitude of the reticulocyte response was not great. Both of these occurred in spite of the fact that the patient received in less than twenty-four hours the extract derived from 8,400 grams of whole mammalian liver. Just what effect the transfusions had on the reticulocyte response is a matter for speculation.[9, 10] Curve 15, Chart 2 represents the reticulocyte curve of Case 15, who received two doses of 50 vials each within a period of forty-eight hours. It is to be noted that Curves 14 and 15 are quite similar. The presence of a low grade, chronic cystitis associated with an hypertrophied prostate and residual urine probably explains

TABLE III

HEMOGLOBIN DETERMINATIONS BY KLETT NEWCOMER METHOD, 100% = 17 GRAMS HEMOGLOBIN PER 100 C.C. BLOOD

CASE	INITIAL LEVEL		MAXIMUM		TOTAL INCREASE		NO. OF DAYS TO MAXIMUM
	RED BLOOD CELLS (IN MILLIONS)	HB (IN GRAMS)	RED BLOOD CELLS (IN MILLIONS)	HB (IN GRAMS)	RED BLOOD CELLS (IN MILLIONS)	HB (IN GRAMS)	
1	2.6	8.0	3.7	10.0	1.1	2.0	21
2	1.2	5.4	4.1	13.5	2.9	8.1	34
3	0.8	3.6	2.0	6.6	1.2	3.0	9
4*	1.5	5.0	1.6	5.0	0.1	0.0	6
5	1.3	4.5	3.7	11.4	2.4	6.9	51
6	3.6	11.8	4.0	11.5	0.4	0.3	16
7	0.8	3.4	2.7	9.3	1.9	5.9	32
8	1.5	6.4	1.9	5.7	0.4	0.7	16
9	1.8	5.4	4.4	13.5	2.6	8.1	35

*This patient left the hospital while the reticulocytes were on the increase.
In some instances the hemoglobin reached a higher level than that of the day when the red blood cells were at their maximum.

the unsatisfactory reticulocyte response of Case 14. It is not entirely certain that the reticulocyte response in Case 15 (Chart 2, Curve 15) is unsatisfactory, in view of the fact that liver had been taken irregularly prior to the time of admission to the hospital.[6] It seems likely, however, that the reticulocyte peak would have been higher had there not been present a low grade pyelitis.

Red Cells and Hemoglobin.—Data concerning the quantitative changes in the red blood cells and hemoglobin are presented in Tables II and III. In general, the figures speak for themselves, but several points seem deserving of comment. In the nontransfused group (Table III), Cases 2, 5 and 9 will be seen to have had the most favorable red cell responses. These cases had an initial level of between one and two millions. Cases 4 and 8 had similar initial levels, but cannot be included in the generalization, the former because of incomplete data, the latter because of the presence of complications. Of the transfused group (Table II), Cases 10, 11, and 12 had their red cells raised to between one and

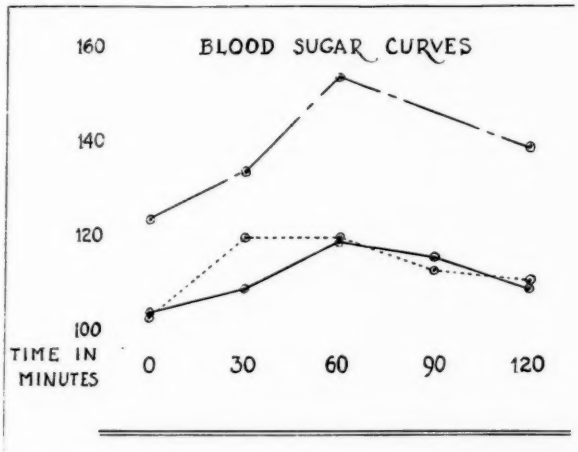

Chart 3.—Blood sugar curves in three representative cases. Blood sugar in milligrams per 100 c.c. blood.

two millions by transfusion. The increase in erythrocytes was not as great in these cases as that seen in a similar but nontransfused group, Cases 2, 5, and 9.

The lack of agreement between the increase of the red cells in these two groups becomes more striking when it is recalled that the original level of the transfused group was below one million. It may be that from the initial level of the red cells one cannot predict the ultimate level to be reached following a single massive dose, as can be done in the case of the reticulocytes.

As would be expected in those cases treated with a single massive dose without transfusion, the greatest increases in hemoglobin were observed in those cases that showed the greatest increases in red cells (Table III). In Case 4 no increase in hemoglobin was noted. Inasmuch as this patient left the hospital at a time when the reticulocytes were on the upward trend, there is no saying what the ultimate level of the red cells and hemoglobin might have been. In Cases 6 and 8 the apparent decreases in hemoglobin might be regarded as coming within the range of the normal fluctuations which are frequently observed in patients under treatment.

What has been said of the hemoglobin in the nontransfused group may also be said of the cases treated with single or repeated massive doses plus single or repeated transfusions, that is, those which showed the greatest increases in hemoglobin were those in which the greatest increases in red cells had occurred (Table II). In Case 12 the occurrence of erysipelas while the red cells and hemoglobin were still on the increase may explain the slight increase in hemoglobin.

White Cells.—In Table IV are given the hemograms of seven cases. Five cases (Cases 2, 5, 7, 9, and 11) showed increased total leucocyte counts during the period of increased peripheral reticulocyte activity. Of these, one (Case 11) showed also a decided shift to the left. A shift to the left was also seen in Case 7,

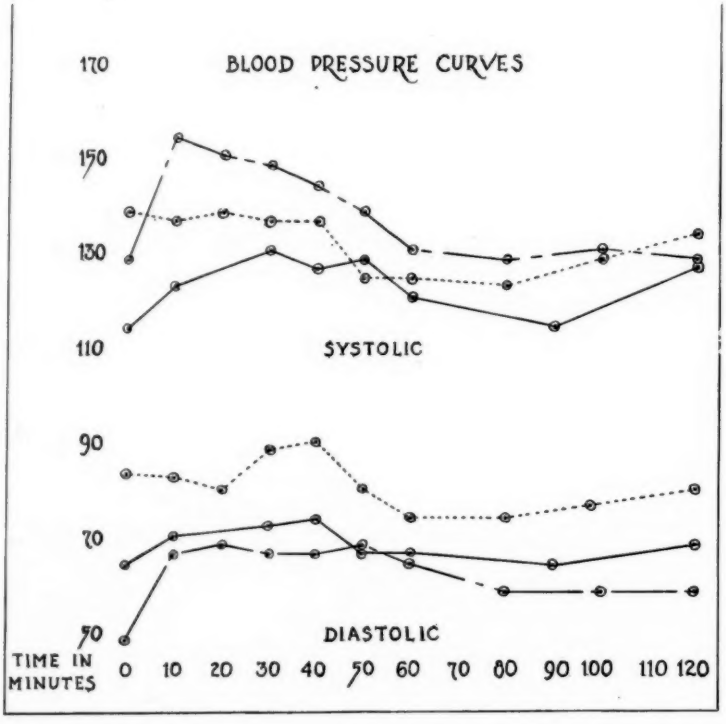

Chart 4.—Blood pressure curves in three representative cases. Blood pressures in millimeters of mercury.

but not associated with any increase in total leucocyte count. Three cases (Cases 2, 5, and 9) showed an increase in polymorpho. ielears with no shift to the left, but associated with a decrease in lymphocytes. One, Case 4, showed a decrease in polymorphonuclears, associated with an increase in lymphocytes, and no change in the total count. Those cases that showed the greatest hematologic and clinical improvement following a single massiv: se of liver extract were those that had the lowest total leucocyte counts befor treatment was given (Table IV, Cases 2, 5, and 9). As already noted, these w.. e also the cases that showed an increase in leucocytes associated with no shift to the left, but with an increase in polymorphonuclears and a decrease in lymphocytes. A distinct shift to the left was noted twice (Cases 7 and 11, Chart 4). These were not the cases that showed the greatest hematologic and clinical improvement in spite of the fact that a

shift to the left means either increased leucoblastic activity, or more rapid delivery of young white cells, or both, and, by implication, increased erythroblastic activity.

Blood Sugar and Blood Pressure.—In discussing dosage and administration, vomiting was noted as occurring in several of the cases. Although it seemed most likely that this could be explained on the basis of local action, in view of the work of Blotner and Murphy[11] and of Althausen, Kerr and Burnett,[12] it seemed worthwhile to determine whether or not the gastrointestinal disturbances might be related to fall in blood sugar, or fall in blood pressure, or both. It is true that the liver fractions used by the above observers were not the same fractions

TABLE IV

TOTAL LEUCOCYTE COUNTS AND DIFFERENTIAL FORMULAE (HEMOGRAMS), BEFORE AND DURING RETICULOCYTE RESPONSE. TOTAL LEUCOCYTES IN THOUSANDS. DIFFERENTIAL FORMULAE IN PERCENTAGE

	BEFORE RETIC. RESPONSE	AFTER RETIC. RESPONSE	BEFORE RETIC. RESPONSE	AFTER RETIC. RESPONSE	BEFORE RETIC. RESPONSE	AFTER RETIC. RESPONSE	BEFORE RETIC. RESPONSE	AFTER RETIC. RESPONSE	BEFORE RETIC. RESPONSE	AFTER RETIC. RESPONSE	BEFORE RETIC. RESPONSE	AFTER RETIC. RESPONSE	BEFORE RETIC. RESPONSE	AFTER RETIC. RESPONSE
TOTAL	2.5	7.0	6.0	6.5	4.8	11.5	5.0	5.1	6.0	12.5	4.0	7.0	8.5	14.0
Myelocytes	0	0	0	0	0	0	0	12	0	0	0	0	0	26
Meta. I (Young)	0	0	0	0	0	0	0	11	0	0	0	0	0	7
Meta. II (Band)	1	1	2	0	2	2	3	9	0	9	2	2	4	4
Polymorphonuclears	29	58	75	38	16	43	89	58	75	73	66	82	74	48
Lymphocytes	67	34	19	58	76	49	8	9	21	8	30	11	17	10
Monocytes	2	6	2	2	2	5	0	1	2	8	2	5	4	4
Eosinophiles	1	1	1	2	4	0	0	0	2	2	0	0	1	1
Basophiles	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Plasma	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Case	2		4		5		7		8		9		11	

that are employed in the treatment of pernicious anemia, nevertheless, we know of no published reports of the effects on blood sugar[13] and blood pressure of such large doses of liver extract as were used in our series. Chart 3 gives three representative blood sugar curves. It is quite apparent that no marked fall in blood sugar occurred. The curves of systolic and diastolic blood pressure in three representative cases are given in Chart 4. Here again it will be seen that no marked fall in blood pressure followed the administration of massive doses of liver extract.

SUMMARY

1. Fifteen cases of pernicious anemia were treated with single or repeated massive doses of liver extract, with or without transfusion.

2. Data are presented on the effects on reticulocytes, red cells, hemoglobin, white cells, blood sugar, and blood pressure.

3. The reticulocytes responded promptly in the complicated as well as in the uncomplicated cases.

4. Two cases showed no reticulocyte response; one because of a previous reticulocyte peak and the other because of the high level of the red cells at the time of treatment.

5. The magnitude of the reticulocyte response seemed to depend on the initial level of the red cells and the presence or absence of complications.

6. Within the limits of the dosage used, the size of the dose seemed to bear no relationship to the promptness of the reticulocyte response, the height of the reticulocyte peak or the duration of increased reticulocyte activity.

7. Transfusion seemed to have no effect on the promptness, magnitude or duration of the reticulocyte response.

8. From the type of the reticulocyte response it was impossible to predict the subsequent course of the red cells.

9. The increase in red cells bore no relationship to the size of the dose given nor to the original level of the red cells, but did seem to be influenced by the presence of complications.

10. The course of the hemoglobin in general paralleled that of the red cells.

11. Increase in white cells was noted in several cases, with or without shift to the left. Increases in some cases were associated with decrease in lymphocytes and increase in polynuclears. In one instance there occurred a shift to the left with no increase in the total number of leucocytes.

12. The occurrence of a leucocytosis, with or without shift to the left, could not be regarded as a favorable prognostic sign.

13. Those cases that had the lowest initial leucocyte counts ran the most favorable course.

14. The administration of 30 to 50 vials of liver extract was without significant effect on blood sugar or blood pressure.

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A SIMPLE APPARATUS FOR THE TRANSFUSION OF BLOOD BY THE CITRATE METHOD*

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TRANSFUSION of blood by the direct method is a most satisfactory operation when performed by a well trained transfusion team. Often, however, the direct procedure is not applicable or possible under many conditions in which transfusion is indicated. Transfusion can never have the wider usefulness which its value as a therapeutic procedure deserves if only the direct method is employed.

The citrate method of transfusion has stood the test of time and is now accepted everywhere as a most satisfactory procedure. Poor technic is probably largely responsible for criticism of the indirect method. I am convinced that blood given properly by the citrate method is fully as valuable as that given by the direct method. The blood should be subjected to the least possible manipulation, and should be obtained and kept in a closed container. The proportion of citrate to blood should be maintained constantly at the optimum concentration (0.25 per cent). The apparatus described herewith fulfills the requirements mentioned above, and has been successfully used by a number of workers in several thousand transfusions.

The apparatus as set up for obtaining blood from the donor is illustrated in Fig. 1. A 500 to 700 c.c. wide-mouth bottle (a 1 pound glucose bottle is most satisfactory), fitted with a rubber stopper is employed. *A* is a stopcock with extension through the stopper to which a rubber tube touching the bottom of the bottle is attached. *B* is a metal sinker carrying a wire strainer. The citrate solution is measured in the barrel of a 20 c.c. Luer syringe. The solution may be run in drop by drop or 10 c.c. at a time. Mouth suction and a 13- to 15-gauge rustless steel needle are used in obtaining the blood. A trap of sterile absorbent cotton, *C*, is placed in the aspirating tube. A slight rotatory movement of the bottle as the blood is running in is all that is necessary for proper mixing. About 100 c.c. of blood should be obtained per minute.

The set-up for giving the blood to the recipient is illustrated in Fig. 2. The syringe barrel has been removed and the inlet and aspirating tubes folded and out of the way. *D* is an automatic stopcock, connected to the stopcock *F*. The blood is withdrawn from the bottle and injected into the vein of the recipient by means of a 20 c.c. Luer syringe. If necessary, air may be expelled or salt solution may be drawn in through the side inlet, *E*. It is necessary, of course, to fill the tubes with blood before connecting them with the needle in the vein

*From the Cleveland Clinic.

of the recipient. I find it more convenient to attach the needle to a syringe, insert it into the vein and then connect the rubber tubing. The blood may be given rapidly or slowly. A few cubic centimeters of blood should be run in and the patient observed for a few minutes for signs of reaction before the remainder is given. Since there is always some drying of blood on the barrel of the syringe, it is usually necessary to use two or more syringes. These are easily exchanged after the stopcock, *F*, has been closed.

Fig. 1.—Drawing illustrating apparatus for obtaining blood from the donor.

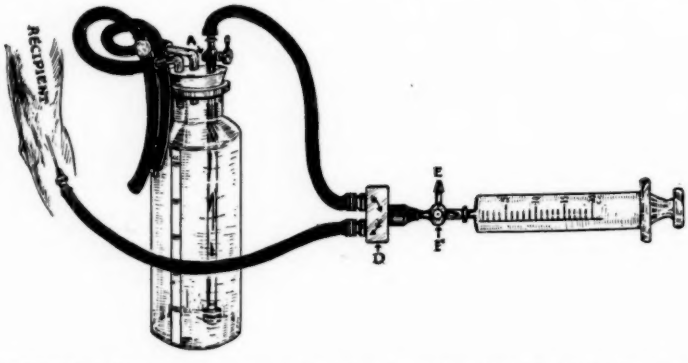

Fig. 2.—Drawing illustrating apparatus for transferring blood to the recipient.

For proper functioning it is necessary, of course, that the apparatus be kept scrupulously clean. The automatic stopcock should always be taken apart after each transfusion and the valve stems and seats carefully cleaned. The component parts of the apparatus, including the rustless needles, are wrapped in towels and autoclaved so that the apparatus is always ready for instant use. I have found it convenient to have several sets with interchangeable parts made up and sterilized so that several transfusions a day are always prepared for.

I have prepared my own citrate solution. A 2.5 per cent solution of chemically pure sodium citrate in 0.85 per cent sodium chloride is put up in

hard glass ampules, which are autoclaved before they are sealed. Ten cubic centimeters of this solution is employed for 90 c.c. of blood which gives a final concentration of 0.25 per cent citrate in the citrated blood.

REACTIONS TO BLOOD TRANSFUSION

Observations From 2500 Transfusions With a Review of the Literature

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ABOUT ten years ago, Karsner[1] stated that the problem of reaction to blood transfusion was by no means solved. Although this statement still holds true today, many of the previously unrecognized phenomena which were responsible for some of the reactions, have since been explained and are now under control. The causes of reactions to blood transfusion, however, are numerous and may be outlined as follows:

I. Incompatibility between donor's and recipient's blood
   This may be due to one of three causes:
   (a) Errors in grouping the blood due to the following causes in order of importance:
      1. Poor technic
      2. Use of low titered or contaminated test serum
      3. Weak agglutinins or agglutinogens in the recipient's blood
      4. Pseudoagglutination
      5. Autoagglutination, cold agglutination and subgroups
      6. Anomalous or atypical agglutination
      7. Contamination of recipient's blood by bacteria
   (b) Indiscriminate use of the universal donor
   (c) Immune isoantibodies and hemolysins
II. The use of unclean apparatus
III. The use of citrate solutions
IV. Incipient coagulative changes in the transfused blood
V. Allergic phenomena in the recipient
VI. Systemic diseases in the recipient
VII. Transmission of disease to recipient

INCOMPATIBILITY

Incompatibility between donor's and recipient's blood is due mainly to errors in grouping, because the tests are performed by internes who, as a rule, do not stay on laboratory service long enough to acquire proper technic. Only the most experienced can entirely avoid the occurrence of such errors. Thus, Lindeman[2, 3] points out that posttransfusion chills occurred in 33 per cent of his cases when he allowed others to do the blood testing, as compared to 7 per cent when he performed the tests himself. Fisk[4] justifiably urges that every hospital have at least one man on its staff who devotes himself exclusively to

this work. Clairmont[5] even goes to the extent of stating that reactions will not occur if the blood groups are correctly determined. Brooks[6] and other writers have expressed similar opinions.

One of the most common sources of error in grouping blood is the use of weak test sera. This may be due to long standing at room temperature or to contamination by bacteria. Dyke[7] has shown that the serum may be considered sufficiently potent, only when it definitely agglutinates its specific isoagglutinogen in dilutions of one to ten. The tests must, of course, be performed on a 2 to 5 per cent cell suspension. More recently, Coca[8] found that test sera offered to the profession by reputable commercial biologic laboratories were below the required standard necessary for grouping purposes. Bacterial contamination which, as a rule, diminishes the potency of test serum, may sometimes be the cause of an error in grouping, due to the agglutinating effect produced by the bacteria or their products. An instance of this type is cited by Grove and Crum[9] who found test serum unreliable because of contamination with mustard bacilli which, in the case cited, clumped cells of Group O, resulting in an erroneous determination of the group. To avoid these errors, test sera should be checked frequently. Weak agglutinogens or agglutinins in the recipient's blood especially in the case of infants[10] may also lead to false group determinations. Failure to recognize this fact has resulted in reactions[11] after repeated transfusion in children in whom the agglutinins developed or became active after the first transfusion. For this reason, Astrowe[12] and others before him have recommended that reliance be placed only on the reaction of the corpuscles and not on the serum, when grouping children under two years of age. In adults, however, the reactions due to such errors are not severe because the agglutinin or agglutinogen which is too weak to be detected in the grouping is also too weak to cause a marked reaction. Thus, in a case previously reported by the present writers[13] in which B blood was given eighteen times to an AB recipient whose agglutinogen A was weak and also in the case reported by Burnham[14] in which A blood was given to a B recipient with weak agglutinin α, the reactions were only very mild or entirely absent. The authors have encountered another similar case in which A blood was injected into a B recipient, apparently without any harmful reaction. On careful reexamination of the blood of the donor by Dr. A. Wiener, it was found that the donor belonged to Group A_2 which is the less sensitive of the two subgroups of Group A. This fact (as in the previous experience of the authors with the Group AB recipient) probably explains the absence of a reaction.

Pseudoagglutination, which is characterized by a rapid sedimentation of the red blood cells, is a phenomenon frequently observed in pregnancy, menstruation, and in certain pathologic conditions (sepsis, tuberculosis, pneumonia, rheumatic fever). These false agglutinations occasionally lead to errors in blood grouping, but can be recognized microscopically by rouleaux formation. It is not an antigen-antibody combination. In contradistinction to agglutinins, the active principle in the serum is not absorbed by treating the serum with red cells.[15] A colloidal, mucoid, or gelatinous substance in the plasma may produce a phenomenon resembling pseudoagglutination. An example of such a substance observed by the present writers is that of Wharton's jelly in the blood of the

umbilical cord. The same observation was made independently by Schott.[16] Pseudoagglutination can be readily avoided by diluting the serum or plasma with physiologic saline in the proportions of one to three. This subject is discussed at length by Shattock,[17] Wiltshire,[18] and Morville[19] and the characteristics of the phenomenon are excellently illustrated in a recent paper by Coca.[20]

Autoagglutination[21, 22] is a phenomenon in which the red cells of a person are agglutinated by his own serum. This may occur normally at ice box temperature (cold agglutination). The clumping is due to a nonspecific agglutinin which acts on cells of all groups. In certain pathologic conditions, however, such as hemolytic icterus, paroxysmal hemoglobinuria, syphilis, and anemia, this phenomenon may occur even at room temperature. Occasionally this leads to error in grouping, because the tyro usually regards these cases as Group AB, although the blood which is being tested may, in reality, belong to any of the four groups. This reaction is recognized by examining a drop of the cell suspension without the addition of test sera, when it will be seen that there is spontaneous clumping of the cells. In these instances correct results can be obtained only by testing the *washed* red cells. *This should be done in all cases in which there is agglutination of the unwashed red blood cells with both A and B test sera.* In contrast to pseudoagglutination, cold agglutination resists slight or moderate dilution and the agglutinins are absorbable by the cells.[23, 24, 25] The action of these agglutinins is diminished by an increased temperature, and in most cases disappears at room temperature. It is never present at body temperature.

Some unexplained reactions may be due to the existence of differences in the blood which are not detectable by the ordinary methods of blood grouping. Thus, differences in the type of A cells have been demonstrated by von Dungern and Hirschfeld,[29] Coca and Klein[30] and others. These have been designated by Landsteiner and Levine[31] as A^1 and A^2. Other differences in agglutinogens have recently been described by Landsteiner and Levine[32] and referred to as M, N, and P. Normal isoagglutinins for M and N, however, have not been demonstrated. Therefore, these agglutinogens play no part in transfusion reactions.[33] It is also still to be determined whether the agglutinins specific to A^1, A^2 or P play an important rôle in reaction to transfusion. Landsteiner, Levine, and Janes have recently reported successful transfusion in 5 cases in which the patients who possessed the agglutinins under discussion, received blood of the same group. These investigators, however, feel that this matter requires further observation. It is therefore still essential to do mutual matching of donor's and recipient's blood in order to avoid any possible reactions which may ensue.

Doan[34] claims that he has seen differences in the leucocytes of individuals of the same blood group and ascribes certain reactions to this phenomenon. These observations could not be confirmed by Rosenberger[35] working in Schiff's laboratory. Further studies on this problem are at present being made by Doan.[36]

Anomalous or atypical agglutinins[26, 27, 28] which are rare, possess intermediate properties between cold agglutinins and typical isoagglutinins. Although they agglutinate red cells irrespective of the group (cells of their own group as well as those of Group O), these agglutinins are, as a rule, active only at room temperature of about 20° C., and very infrequently at 37° C. They therefore

rarely lead to errors in grouping. Experience, however, has shown that even this possibility must be kept in mind.

If the blood which is drawn from the recipient for grouping is not fresh (after twenty-four or more hours at room temperature), the red cells may become agglutinable by all human sera, thus causing a wrong determination of the group to which the patient belongs. Thomsen and Friedenreich[37] have shown this effect to be due to bacterial products. As a rule, however, suspensions of fresh blood are used for grouping, so that this is rarely a cause of difficulty. Lacy[38] also found that in cases of bacteremia the serum may contain agglutinating substances due to the infecting organism.

The changes in the blood group which Levine and Segall[39] claim to have found in patients who were subjected to prolonged etherization as well as those found by Eden[40] to be due to certain drugs (quinine, antipyrene) and by Benda and LeClerc[41] to be due to radio therapy, are probably all based upon erroneous observations, for it is now well known that the isoagglutinogen and isoagglutinin of the blood remain permanent after the second year of life. Landsteiner,[42] the discoverer of isohemagglutination, is strongly of the opinion that a blood group, once established, never changes.

"Universal donors" are used as such by many on the grounds that the red cells of this group are not agglutinated by any human serum. Thus, Brines,[43] in a recent paper, advocates as a safe procedure, the routine use of the "universal donor." Exception must be taken to this statement because of the not infrequent reports of alarming and even fatal reactions occurring as a result of such indiscriminate use of these donors. Unger[44] has reported several such observations and Copher[45] mentions the occurrence of fatal accidents following such practice. The dangers involved in the routine use of the "universal donor" have also been emphasized by the Inter-allied Surgical Congress in a recent report. In 1926, Freeman[46] reported the case of a universal donor who possessed an "alpha" agglutinin which was so powerful, that if he had acted as a donor for the other groups (A or AB), according to the author, the results would have been very serious. Similar dangers had been previously pointed out by Levine and Mabee.[47]* Gray,[48] in a review on the results of five hundred transfusions, in which donor and recipient were of the same group, found that there were only 10 per cent reactions as compared to 33 per cent of forty transfusions in which universal donors were used. Landsteiner is of the opinion that donors of the homologous group are preferable and also states that the clinical experience of Jones, Glynn, Kubanyi, Butka and Kraft support this view.

In the last decade there have been observed posttransfusion reactions which were apparently due to the development of isoantibodies in the plasma of persons who had previously received injections of compatible blood. In connection with this phenomenon, the following may be quoted from Landsteiner's chapter on the human blood groups[26]: "This consideration may have a bearing on the results of repeated transfusions in man, in view of the possible formation of immune isoantibodies in human beings, in analogy to experiments in animals.

*Since writing this paper, L. W. Parr and H. Krischner reported a case of a fatality from a transfusion in which both the donor and recipient belonged to Group O (Hemolytic Transfusion Fatality With Donor and Recipient in the Same Blood Group, J. A. M. A. 98: 47, 1932).

In fact some cases were reported in which blood clinically compatible at the first transfusion caused disturbances when used repeatedly. This phase as well as the findings of abnormal isoagglutinins after several transfusions requires further investigation. . . . . There is little doubt that transfusion could incite the production of isoantibodies when incompatible blood is injected which is tolerated at first by virtue of low agglutinin content of the recipient's serum." This appears to be substantiated by one of the cases reported by Astrowe. In this case the recipient (a child eighteen months of age) belonged to Group O, but received blood from its mother who belonged to Group A. The first transfusion was well tolerated because the isoagglutinin had not yet become active. A subsequent transfusion from the same donor, however, resulted in an almost fatal hemolytic reaction accompanied by severe shock. Apparently the first transfusion incited the production of isoantibodies which were responsible for the reaction, or it might have been that these isoantibodies became active in the interim between the first and second transfusion. Previously, Lindeman[49] also cited a case in which an hemolytic reaction occurred five days after a second transfusion of blood from the same person who acted as donor in the first transfusion, which was uneventful. Subsequent tests showed hemolysis of donor's cells by the patient's serum. More recently, Landsteiner, Levine and Janes[50] reported a case in which an abnormal isoagglutinin was found in the serum of a patient after the first and second transfusion and which reacted on numerous blood specimens, including cells of Group O, and those of the donor. In this case, however, there were no posttransfusion reactions. Hemolytic reactions even after the first transfusion of compatible blood have also been observed. Carrington[51] cites a case in which the recipient developed hemolysis soon after a transfusion of blood from a donor who had been previously tested and found to be compatible. It is quite certain that such instances, although not frequently reported in the literature, are not of infrequent occurrence.

UNCLEAN APPARATUS

Unclean apparatus is known by all who perform blood transfusions to be frequently the cause of reactions. This may be due to the powder of new rubber tubing, accumulated blood clots in the apparatus employed (especially if the latter is a complicated one), debris which remains in the apparatus or needles after sterilization, etc. The tendency recently has been to simplify the apparatus with the aim of eliminating the possibilities of such collections of extraneous matter.[52, 53] Passing saline through the apparatus just before the transfusion will prevent accidents of this type.

CITRATE

Since the demonstration by Agote[54] in 1914, and the independent observations by Weil[55] and Lewisohn[56] in 1915, that sodium citrate may be used as an anticoagulant in blood transfusions, there has arisen a great controversy regarding the advantages and disadvantages of the use of citrate intravenously. Much has been written pro and con. In 1917, Sydenstricker[57] found only 17 per cent posttransfusion reactions when citrate was used. Ravdin and Glenn[58]

from a study of 161 citrate transfusions, concluded that the simplicity of this method warranted its preference over others in vogue at that time. They encountered as many chills with the use of paraffin glass cylinders as with sodium citrate. In a similar review of 269 cases studied by Lewisohn[59] the latter concluded that citrate transfusions were as satisfactory as any other method employed at that time, which at best were crude as compared to the perfected methods of today. As regards the production of hemolysis by citrate, O'Malley and Hartman,[60] in the light of their work on citrated plasma in the treatment of pneumonia, concluded that the hemolysis is due not to the sodium citrate but to contact of the blood with the wall of the container, resulting in trauma to the platelets with subsequent thrombin formation. In a subsequent publication, Hartman[61] reiterated this point and cited the experiments of Minot[62] and the observations of De Kruif[63] to substantiate this opinion.

The general toxic effect of citrate in the blood stream is also a controversial question. Henderson,[64] in a letter to Joannides[65] who, in 1924, was investigating this problem, agreed with the latter that the use of citrate in such small quantities as are employed in blood transfusions is not dangerous. Gichner[66] found that citrate is not toxic when used in even larger doses. Rous and Turner[67] furthermore, were of the opinion that not only is citrated blood harmless but even of particular advantage during war time when it becomes necessary to have a constant supply on hand, since it is possible to preserve the blood for a month if prepared in the form of a mixture of dextrose and sodium citrate (3 parts of blood, 2 parts of 3.8 per cent sodium citrate and 5 parts of 5.4 per cent dextrose in water). Hoffman[68] also considered citrate transfusion a useful procedure and believes that the deleterious effects and even the fatalities which have been ascribed to citrate are not due to citrate per se, but to other causes, such as poor technic, bad risks, etc.

There are, however, many observers who decry the use of citrated blood. Thus, Brines[69] states that there is never a time when citrate is as good as whole blood. Bacon[70] deprecated the use of citrated blood, asserting that it carries with it "heavy liabilities." Bernheim[71] has been even more emphatic in his warning, an abstract of which follows: "It was thought that increased familiarity with this method will decrease the evils. . . . . The dread reactions still persist, and persist despite the most painstaking efforts to discover their cause or origin, despite the most careful mixing of citrate with blood, despite the most carefully planned and executed citrate transfusions carried out by men whose long experience with general blood transfusions would preclude the possibility of technical error." After citing several fatalities, as a warning to others, he continues, "So I maintain that this reaction following the citrate transfusion with its inherent danger and failure of medical men to take cognizance of its importance, is analogous to the story of our erstwhile indifference to blood tests, and I am trying to sound a warning." It has also been charged by Unger[72, 73] and others that citrate destroys platelets, that it develops anticomplementary properties in plasma; that it reduces the phagocytic and opsonic index of the blood; that the red blood corpuscles are made more friable, and that it produces a general systemic reaction with malaise and chills. That such reactions are common is apparently borne out by the observations of numerous

investigators. Kretzler[74] found that reactions are more frequent when citrated blood is used than when even so crude a method of unmodified blood transfusion as that of Lindeman is employed. Pauchet[75] encountered disagreeable chills with citrated blood and was forced to resort to a method in which paraffin coated syringes were used instead of citrate. Platt,[76] Moons[77] and Hermann[78] have each described blood transfusion fatalities which they believed to be due to the use of citrate. Bernheim[79] found that despite precautions, reactions occurred in 20 to 40 per cent with refined methods of whole blood transfusion. Meleney et al.[80] encountered reactions in over 64 per cent of 196 cases in which citrate transfusions were performed. Lederer,[81] in a comparative study of a consecutive series of 100 transfusions of citrated and whole blood transfusions, encountered about 50 per cent reactions with citrated and none with whole blood transfusions. More recently, a similar study conducted by Landon[82] revealed that reactions occurred five times as frequently with citrated as with whole blood.

The exact causes for these reactions are still unknown. According to McClure,[83] they are due to the prolonged exposure of the blood to foreign substances that the citrate method necessitates. The experimental investigations and extensive practical experience of Drinker and Brittingham[84] have led them to conclude that the reactions are due to the deleterious effects of the citrate on the blood platelets. Horsely et al.[85] are of the opinion that citrate produces certain chemical and biologic changes in the blood, which changes may account for the reactions. Whichever is the correct explanation, it is quite obvious from the review of the literature, that reactions are much more common with citrated than with unmodified blood transfusions.

INCIPIENT COAGULATIVE CHANGES

Reactions, however, occur with none of the aforementioned causes to explain them. Some of these resemble the reactions which are due to hemolysis or agglutination, yet present no evidence of the existence of either of these phenomena. Such reactions have been ascribed to the incipient coagulative changes in the transfused blood before it enters the circulation. From a study of the effects of physical influences on blood, Satterlee and Hooker[86] have been led to conclude that such altered blood contains potential coagulative factors, such as thromboplastin and thrombin, which probably produce coagulation after reaching the circulation, yet the changes are too fine to be detected by the ordinary methods. Similar views are held by Clough,[87] Horsely[88] and others. For want of a better explanation the above must be accepted as accounting for this type of transfusion reaction. The experienced transfusionist who has seen the frequently unavoidable agitation, whipping and other abuses to which the blood is exposed before it is injected into the recipient's vein, can best appreciate the logic in such an explanation.

ALLERGIC REACTIONS

Allergic reactions to blood transfusions are not uncommon. Carrington[89] reports a case in which the patient, within ten hours after receiving 50 c.c. of compatible citrated blood, developed dyspnea, laryngeal stridor, cyanosis, pul-

monary edema, became comatose, and died. There was no hematuria or hemoglobinuria. Blottner[90] cites several instances of shock from repeated transfusions of compatible blood. The nature of these reactions, the presence of eosinophilia, and the fact that the reactions in these cases occurred with those transfusions which were performed in three to six weeks after the first, indicated that they were allergic in nature. In reviewing the results of 700 transfusions, Duke[91] states that allergy is an important contraindication. The allergic phenomena, according to Hanzlik,[92] depend upon a disturbance of the physical and chemical (colloidal) mechanism of the blood and tissue. According to Kordenat[93] most of the so-called "anaphylactic reactions" are really due to protein shock, which may occur in one of two ways. First, the donor's blood may increase the protective or cleavage power of the patient's serum so that there is cleavage of damaged tissue which produces the reaction. Secondly, because of poor proteopexic ability of the liver, partly altered proteins leak through, resulting in parenteral cleavage and nonspecific protein shock (Widal crisis). Anaphylactic reactions have occasionally been ascribed to food allergy. Thus, Duke and Stofer[94] encountered reactions in sensitive persons who received blood from donors who ingested the offending food before the transfusion. This theory has been partially substantiated in a report by Brem[95] who claims that such reactions were prevented by using fasting donor's blood. Hustin[96] believed that anaphylactic reactions to foreign protein can be warded off by preliminary injections as described by Besredka. In our experience, adrenalin has been found extremely helpful in warding off and combating most of the so-called allergic reactions.

In concluding the very brief review on this phase of the subject, it may be stated that the information thus far gathered is very unsatisfactory. According to the most recent opinions held by the foremost investigators of hypersensitiveness, it is questionable whether the reactions described above are truly allergic. Urticaria, eosinophilia, the effect of adrenalin in controlling the symptoms, are not necessarily signs of an allergic phenomenon. It is not within the scope of this paper to delve into the intricacies of the subject. For a comprehensive discussion of this phenomenon, the reader is referred to the works of Coca and to those of Welch and Karsner.[97]

SYSTEMIC DISEASES

The transfusion of blood may have deleterious effects on the kidney with resultant severe and frequently fatal reactions. An excellent review of this subject was recently written by Boardley[98] who described 3 cases of this type, 2 of which were fatal, and also cited about 22 other cases previously reported by other authors. In sixteen of these the reactions were fatal. According to Brines, the reactions occurring in these persons were due to underlying renal disease. Thus Schumacher[99] reported 2 cases in which blood transfusion precipitated fatal uremia in patients who were suffering from previous renal impairment. A greater number of the cases previously mentioned, however, showed absolutely no evidence of renal impairment before the transfusion. We have also found the converse to be true. Many patients with nephritis, some

of them with marked retention, have received transfusions without any untoward results.

A review of the literature on this point confirms this observation. Ottenberg[100] carried out a large number of transfusions on patients with diseased kidneys, and several with one kidney, with no untoward results in any. Mosenthal[101] failed to notice a suppression of urine after transfusions on patients with Bright's disease. He believed that anaphylaxis and hemoglobinemia from incompatible blood cause the damage to the kidney, but that the effect is the same in the normal person as in those with Bright's disease. On the contrary, favorable results, following transfusion in cases of renal disease, have been reported by Ramsay,[102] Martin,[103] Iversen,[104] and Flandin.[105] In nephrosis likewise, no contraindication to transfusion has been reported. Epstein,[106] Clausen,[107] and others failed to find any unfavorable results in these cases, and in fact recommend it as a valuable therapeutic measure.

The cause of the renal involvement is still not understood, and is an extremely interesting problem. Boardley believes that it is all due to incompatible blood, although definite incompatibility was established in only 6 of the 17 cases which he describes in more or less detail. One of his own fatal cases, furthermore, received blood which was found by previous cross agglutination tests to be compatible with the recipient's blood. One of us (P) also had an experience with a fatal case in a patient of this type who received blood which, as far as could be determined by the usual methods of cross agglutination, was compatible with that of the recipient. To ascribe all reactions of this type to incompatible blood would, therefore, not account for these rarer instances, and a more satisfactory explanation is still wanting. The anatomic changes in these cases have as yet offered little information in that respect. The outstanding changes in the kidneys consist of swelling and advanced degeneration of the tubular epithelium (many of which contain a peculiar brownish pigmented material which is believed by some to be of hemoglobin origin). Masses of blood pigment, as well as débris, leucocytes, wandering cells, and desquamated cells are found in the lumina of the tubules. Dilatation of the glomerular capsules and an interstitial cellular infiltration occur in some of these cases. In the liver, central necrosis is the common finding.

The presence of the masses of hemoglobin pigment in the renal tubules is the basis for the theory that the blockage of the tubules by these casts produces a renal insufficiency. But, as has been definitely shown by Sellards and Minot[108] and later by Rich,[109] the injection of hemoglobin into human beings failed to produce renal impairment. At this point, it may be interesting to cite the experimental evidence of Baker. He showed that in these cases hemoglobin is thrown out in solution when the P_H of the medium is 6 or less and when the sodium chloride content is 1 per cent or over. Under such circumstances, he claims, hemoglobin is excreted in solution in the glomerular transudate and after concentration in the tubules, the pigment is precipitated in the form of hematin, due to the acidity and increase in salt concentration. According to Baker, this condition may, in fact, be combated by producing alkaline diuresis.

The changes noted in the tubules of the kidney, which are so similar to those of the so-called tubular nephritis, described by Brown et al.[110] as occurring in

pyloric obstruction with vomiting, has lent support to the theory that the post-transfusion renal insufficiency follows a loss of chlorides incident to vomiting and its consequential nitrogen retention.

Acute poisoning by an irritating or toxic substance as being a cause of post-transfusion renal insufficiency is suggested by the evidences of inflammatory reaction observed in the kidney (leucocytic infiltration, edema, necrotic tubular epithelium), and also by the foci of necrosis in the liver.

From a clinical point of view, the nature of the symptoms of the reaction favors some and negates others of the theories mentioned to explain the causative factors. Thus, the theory of toxic irritation is strengthened by the similarity between the symptom complex of black water fever and that of the transfusion reaction, while the theory of hemoglobin blockage of the tubules is weakened by the absence of hemoglobinuria in some of these reactions. The similarity between the reactions to transfusions and shock which is frequently associated with severe renal insufficiency, as pointed out by Longcope and Rackemann,[111] has led to the consideration of a theory which ascribes the reaction to shock resulting from a kidney sensitiveness to incompatible blood. This phase has already been considered in the discussion of allergic reactions.

Other organs besides the kidneys may be overtaxed by blood transfusions. As Wildegans[112] recently emphasized, the heart, liver, and blood vessels may all suffer serious injury. Bisenberger[113] reported a case of sudden cardiac paralysis after the injection of only 150 c.c. of blood. This was a case of mitral stenosis in which the injury to the heart was proved at autopsy. Probably numerous other fatalities have occurred due to overtaxing of the heart, especially in those persons whose cardiac reserve is at a low level. In this connection, Eyster's[114] experiments are of interest. From an x-ray study of the silhouette area of the heart he found that in the recipient, increments of blood by transfusion in amounts to within 1 per cent of body weight resulted in a transitory alteration of the cardiac size. Although it was found that the compensatory mechanism in the cases studied, caused a rapid adjustment to the normal circulatory conditions, not withstanding the altered blood volume, it is questionable whether a diseased heart would show a similar compensatory ability. We have frequently observed a severe type of reaction which is most probably due to overdistention of the right side of the heart, with what appears to be an interference to the proper return flow of the cerebral circulation. That the reaction is cardiac in nature, is evidenced by the fact that the patient suddenly becomes pulseless and cyanotic, and the cardiac sounds become almost inaudible. At the same time there is a loss of consciousness, rolling of eyeballs, and in some instances muscle spasms. This is soon followed by cold and clammy perspiration and then simultaneously with the cessation of the transfusion, a gradual return to consciousness. The patient "wakes up" from a brief "sleep." All this transpires in a period of a minute or two, provided the symptoms are recognized early enough and the transfusion is halted. Failure to observe the onset of this reaction quickly enough may prove fatal to the patient, or result in a prolonged reaction, so that unconsciousness may persist for as long as ten or fifteen minutes, and a much slower return of the heart action to normal. This type of reaction occurs in persons with poor myocardial tone. In performing trans-

fusions on such patients, therefore, it is extremely important, to watch the pulse and to inject the blood slowly and guardedly.

TRANSMISSION OF DISEASE

The transmission of disease from donor to recipient and vice versa, may be considered a reaction to blood transfusion. This hazard has recently been pointed out by a number of observers. Transmission of asthma has been reported by Ramirez[115]; measles, by Baugess[116] and Harrell[117]; smallpox, by Blalock[118]; malaria, by Korabelnikoff,[119] Flaum[120] and others; and syphilis, by a number of observers including ourselves.[121-132] It may be added that syphilis has similarly been transmitted to the donor. In order to avoid these complicating reactions, rigid physical and serologic examination of family as well as professional donors has been urged.

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SPECIAL FEATURES OF THE BLOOD IN INFANCY AND CHILDHOOD*

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IN INFANCY and childhood the hemopoietic system undergoes changes which lead to the adult condition. The bone marrow produces red and white blood cells side by side, and fills all of the bones. It is only with onset of adolescence that the red bone marrow begins to recede from the shafts of the long bones and be replaced by fat. Just as the red bone marrow may replace this fat during severe anemias in adults and thus revert to the childhood type, so the bone marrow of infants more easily reverts to the fetal type under the stress and strain of hemopoietic stimuli or irritants. This apparent difference in response to similar disease processes has given rise to the impression that blood dyscrasias of children are quite different from those of adults and represent separate disease entities. There are additional features of the normal infant's bone marrow and blood, an appreciation of which is important in evaluating the different blood pictures.

Anatomy of the Bone Marrow.—The anatomy of bone marrow varies considerably at different age periods. Piney[1] pointed out that at birth and for the first three to four years, the cut section of the marrow is pink, and the expressed tissue a rich red. At the age of seven years the marrow is less pink and the surface appears greasy. The expressed tissue has distinct fat droplets; however, no macroscopic areas of fat are visible until the age period of twelve to fourteen years. At this time fatty patches occur at the middle of the shafts of the long bones. Within twelve months other smaller fatty areas are noted throughout the marrow. These same changes occur in the tibia, fibula, femur, radius, ulna, and humerus. The fatty changes occur much more rapidly in the lower extremities and, further, these changes are more rapid in the distal portions of the bone than in the proximal. When the tibia and fibula are completely filled with fat, the upper end of the femur still retains red marrow. This is the adult picture as it exists in the long bones. The epiphyses also become entirely fatty by a similar process as previously described. Red marrow is always to be found in the ribs, sternum, vertebrae, os innominatum, and in the bones of the skull.

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The adult marrow has a volume of about 1400 c.c., which is far in excess of the actual amount required for maintaining a normal physiologic function. As a result, a large reserve space is present which is potentially available for the formation of blood cells under abnormal conditions. The marrow of the infant lacks this reserve. For the body of the infant to compensate for an anemia it has two mechanisms: 1, the displacement of fluids, blood and plasma; 2, the resorption of bone. These two processes safeguard the function of the marrow under normal conditions and to a certain extent under pathologic conditions. However, with increasing abnormal demands which the adult could care for by his "reserve," the infant is unable to compensate for and the marrow suffers as a result. The added load is too great, and the immature cells of the marrow are thrown out into the stream long before they are ripe, giving the typical embryonic type of peripheral blood. In severe anemias, foci of blood formation may reappear in the liver and spleen. The blood picture of anemia in the adult after a prolonged period of time may simulate that of the infant, but the immediate pictures differ, since the adult has the mechanism of "reserve."

Hemoglobin in Infancy and Childhood.—The majority of the reports in the literature concerning the hemoglobin estimations of infancy and childhood are usually given in "per cent" with no data as to the value of 100 per cent in absolute figures. Some authors have given average figures for the various age groups, omitting the number of cases studied. In Table I are listed the average

TABLE I

AGE	AMOUNT OF HEMOGLOBIN GRAMS PER 100 C.C.	PERCENTAGE OF HEMOGLOBIN (14 GRAMS = 100 PER CENT)	NUMBER OF CASES
1 Day	19.23	137%	103
2 Days	16.23	116	40
2-3 Days	22.05	158	45
3 Days	15.82	113	30
4 Days	16.19	116	39
4-8 Days	20.99	149	47
5 Days	15.90	113	34
6 Days	15.43	110	35
7 Days	15.29	109	29
8 Days	14.26	101	34
9 Days	14.30	101	29
10 Days	12.50	89	26
11 Days	13.83	99	31
12 Days	12.74	91	12
13 Days	17.40	112	1
9-13 Days	20.89	149	38
24 Days	14.40	103	1
14-56 Days	17.00	121	46
3-5 Months	13.30	95	42
6-11 Months	13.30	95	43
12 Months	12.57	89	34
11-23 Months	11.90	85	12
2 Years	12.57	90	33
3 Years	13.16	94	31
4 Years	13.62	97	31
5 Years	13.54	97	35
12 Years	11.06	79	7
13 Years	11.20	80	33
14 Years	11.48	82	17
15 Years	12.32	88	27
16 Years	12.32	88	13

hemoglobin content in grams per 100 c.c. and in equivalent percentages (using 14 grams as 100 per cent) of 777 healthy children collected from the literature and 100 cases observed by the author. The periods covered are from birth to the fifth year, and from the twelfth to the sixteenth year.

For the first ten days the hemoglobin ranges from 22.05 grams to 12.5 grams per 100 c.c. of blood with the average well above that found in adults. Gradually it falls to a level of 11.9 grams, being the lowest from the third month to the second year. There is then a slow return to the adult range with a slight drop again at puberty.

Red Blood Cells.—The number of red blood cells present in the peripheral blood seems to parallel the amount of hemoglobin. At birth, and for the first few weeks following, the count may be as high as seven million red blood cells per cubic millimeter, the average being about five and a half million per cubic millimeter. From the first month to the sixteenth year, the average count is approximately four and a half million per cubic millimeter. The results com-

TABLE II

AGE	RED BLOOD CELLS MILLIONS PER CU. MM.	NUMBER OF CASES	
Birth	6.83	87	(Umbilical cord cut immediately— 6 cases 5.08 Umbilical cord cut after arterial pulsations cease— 8 cases 5.57) Hayem
½ Hour	5.19	71	
6 Hours	5.66	71	
11 Hours	4.57	1	
12 Hours	5.52	71	
18 Hours	5.38	71	
24 Hours	5.65	155	
30 Hours	5.37	71	
36 Hours	5.43	71	
48 Hours	5.43	110	
3 Days	5.29	30	
1-3 Days	4.83	30	
4 Days	5.43	38	
5 Days	5.21	31	
6 Days	5.14	34	
7 Days	5.15	29	
8 Days	4.73	33	
9 Days	5.36	36	
10 Days	4.65	26	
4-10 Days	4.65	30	
11 Days	4.73	31	
12 Days	4.50	12	
13 Days	3.69	1	
14 Days	5.79	10	
24 Days	3.40	1	
3 Months	4.50	17	
1 Year	4.00	10	
2 Years	4.50	10	
5-10 Years	5.00	10	
12 Years	4.66	7	
13 Years	4.68	33	
14 Years	4.77	17	
15 Years	4.75	27	
16 Years	4.66	13	

piled in Table II have been collected from data on 1,198 healthy children obtained from the literature and 100 cases investigated by the author.

At birth and the following few days, nucleated red cells are present in decreasing numbers. Neumann[2] believed that they are common in the newborn. Fischl[3] found them present in one case, and absent in two. Hayem and Luzet[4] claim that erythroblasts disappear from the blood of the fetus during the later weeks of pregnancy, while Hock and Schlesinger[5] found them present in healthy children. Most authors believe that nucleated red cells disappear by the fourth day. At birth the reticulocytes vary from 5 to 10 per cent (Friedlander and Wiedemer,[6] Seyforth and Jürgens[13]). Anisocytosis, poikilocytosis, and polychromatophilia are also marked in the blood of the newborn. The various signs of regeneration assume normal proportions within a few days. In addition, some authors have reported the presence of the so-called "ghost cells" at birth. Their significance is not known.

White Blood Cells.—In the newborn, the number of white blood cells present in the peripheral blood is markedly elevated. It may total as high as forty thousand per cubic millimeter, the average being twenty thousand per cubic millimeter. Up to the eighteenth hour, there is a slight increase in the total number of white cells, followed by a gradual decrease. (This high white cell count probably includes the nucleated red blood cells which are present.) All during infancy and early childhood a slight leucocytosis is present, the average number of cells being 12,000 to 14,000 per cubic millimeter. About the tenth year the normal, adult count is reached. In Table III are listed the averages of the various age groups.

TABLE III

AGE	TOTAL WHITE BLOOD CELLS PER CU. MM.	NUMBER OF CASES
Birth	18,100	58
½ Hour	16,600	71
6 Hours	21,000	71
12 Hours	22,500	71
18 Hours	21,200	71
24 Hours	17,700	130
30 Hours	17,600	71
36 Hours	15,400	71
48 Hours	12,200	143
72 Hours	12,900	57
96 Hours	10,100	76
5 Days	9,120	61
6 Days	11,200	61
7 Days	11,500	38
8 Days	11,700	58
9 Days	12,100	57
10 Days	12,400	45
30 Days	12,200	18
2 Months	13,500	3
3 Months	13,200	2
4 Months	11,200	12
5 Months	13,200	2
6 Months	13,000	2
1-2 Years	6,500	150
2-3 Years	7,350	150
3-4 Years	6,400	150
4-5 Years	6,560	150

There have been many theories presented in explanation of the high leucocyte count. Some authors believe it to be due to the loss of body fluids; others as due to trauma during delivery; while still others believe it the result of bone marrow stimulation, or a reaction from ingested protein after eating for the first time.

Platelets.—Of all the cellular elements derived from the bone marrow in the peripheral blood, the platelets are the only ones not increased in number. They average from two hundred to four hundred thousand, and this amount remains practically constant throughout life.

In addition to the theories presented in explanation of the leucocytosis, several others have been offered to account for the increased numbers of red cells, hemoglobin percentage, and white cells of the newborn. Lepine[7] believed the variation due to changes in the plasma volume; Hayem[8] accounted for the increased numbers as the result of a new formation of the elements. In addition, he demonstrated a variation in the number of red blood cells when the umbilical cord was cut immediately and after waiting for the arterial pulsations to cease. In fourteen observations there were approximately one-half a million red cells per cubic millimeter more in samples of blood obtained after the pulsations ceased. Schiff[9] believed the blood to be concentrated as a result of the loss of body fluids. Elder and Hutchinson[10] are inclined to agree with Hayem, for the figures observed varied too much within a few hours to be accounted for by either increased cell production or hemolysis. If the bone marrow were stimulated as the result of lack of oxygen or increased body need, or if the blood were concentrated from the loss of fluid, the platelets should also be increased in amount.

The presence of immature red blood cells and white blood cells in the peripheral circulation for only a few days, in addition to the fact that the platelets remain constant, leads one to account for the newborn blood picture by some other method. This has been suggested by Schilling,[11] and it is thought to be clearing of the liver and spleen as foci of hematogenesis.

Differential (Leucocyte Count).—During the first twenty-four to forty-eight hours, the polymorphonuclear leucocytes are present in abundance, assuming the proportion seen in adult life. After two or three days, they decrease from a total of about 60 per cent to 35 or 40 per cent. The eosinophils average 3 to 7 per cent, while the basophils remain about 0.5 per cent. The mononuclears average 3 to 10 per cent. The difference is made up with small and large lymphocytes. The types of cells present after the first few days are all adult in nature. This factor assumes great clinical value when estimating the importance of the blood as a means of diagnosis. Table IV is a summary of the average differential counts of many observations previously reported.

Smith[12] has investigated the presence of lymphocytosis in 37 infants, 21 of whom were less than one year old. He made comparative differential counts in living preparations and stained smears. The percentage of polymorphonuclear leucocytes was 8.6 per cent more in the fresh preparation than in the fixed film; and further, the number of lymphocytes in a dry smear was 11 to 14 per cent more than those in a living sample. This difference he believes is due to the failure of the observer to identify properly the mashed cells present in the coverslip preparations and the unequal distribution on the Wright

TABLE IV

AGE	POLYMORPHONUCLEAR NEUTROPHILS %	EOSINOPHILS %	BASOPHILS %	LYMPHOCYTES %	MONOCYTES %
½ Hour	50.4	2.2	0.2	44.5	2.3
6 Hours	59.1	1.4	0.2	36.4	2.7
12 Hours	66.0	1.2	0.2	30.1	2.6
18 Hours	63.6	2.0	0.1	32.0	2.0
24 Hours	60.6	2.6	0.2	29.5	4.2
36 Hours	51.8	3.0	0.1	42.8	2.2
48 Hours	54.9	2.1	0.2	35.0	5.3
72 Hours	49.0	2.4	0.2	39.0	7.5
96 Hours	43.5	2.4	0.2	45.0	8.1
5 Days	41.0	3.0	0.2	46.0	9.0
6 Days	37.0	3.8	0.1	50.0	9.5
7 Days	35.0	4.5	0.15	52.5	9.5
8 Days	24.5	2.8	0.15	63.0	10.0
9 Days	31.5	4.3	0.0	55.5	10.5
10 Days	26.0	1.9	0.25	63.0	9.2
11 Days	26.0	1.7	0.25	61.5	10.5
12 Days	30.0	2.0	0.5	62.0	5.5
2-14 Days	32.9	6.2	0.2	52.0	10.1
2-4 Weeks	28.7	4.9	0.4	56.3	9.0
1-2 Months	29.8	6.1	0.46	53.8	12.0
1-6 Months	34.5	3.5		50.0	11.0
2-6 Months	26.3	2.6	0.30	60.0	10.0
6-12 Months	35.0	1.5	0.3	52.0	9.5
1-2 Years	41.9	3.0		47.0	4.0
2-3 Years	48.2	3.9		38.4	4.5
3-4 Years	52.6	5.7		33.2	4.2
4-5 Years	61.0	6.3		25.8	3.7

stained films. With the above correction, the differential count seen in infancy and childhood would more closely approximate that of the normal adult. This would mean, however, that polymorphonuclear neutrophils in babies were more fragile than those of the adult and that the physical properties of their blood were also different.

Discussion.—A review of the various blood pictures described indicates a slow transition from the blood picture of infancy and childhood to the normal picture of the adult. However, there are several features of the bone marrow and blood of infancy and childhood which are outstanding: 1, the distribution of the bone marrow; 2, the changes in hemoglobin values; 3, a polycythemia at birth and for about one month following; 4, a persistent leucocytosis; 5, the presence of immature red and white blood cells in the peripheral blood during the first few days of life; 6, lymphocytosis up to the second or third year.

In addition to the above-mentioned features is the abnormal response of the bone marrow in infancy on the slightest provocation with a reversion to the embryonic type of blood. Infection in adults is manifested by a leucocytosis, whereas in infants, not only is a leucocytosis present, but many immature white cells are thrown into the stream with a resulting leucemoid picture. Anemias in adults, unless severe, rarely if ever show immature red cells in the peripheral blood, because of the "reserve" of the bone marrow (the replacement of fat marrow by red marrow). In infancy and childhood there is no "reserve" of the bone marrow, and any extra demand results in an overactivity which furnishes only increasing numbers of immature red cells to the blood. Normoblasts,

megaloblasts, anisocytosis, poikilocytosis, and polychromatophilia are much in evidence. The liver and spleen often assume the rôle of hemopoiesis which they formerly had in the fetus. The understanding of this vague mechanism is most important in evaluating the confusing blood pictures occurring in infancy and childhood.

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DIAGNOSTIC FEATURES OF THE BLOOD COUNT AND OF THE MORPHOLOGY OF THE BLOOD IN DISEASES ASSOCIATED WITH SPLENOMEGALY*

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PHYSICIANS intimately concerned with clinical hematology have long recognized minor differences in blood counts which they have more or less intuitively used as aids to diagnosis; however, these minor characteristics have rarely been analyzed. Likewise, hematologists interested chiefly in examination of the blood have described many variations from the normal, which, however, have not been sufficiently simplified to come within the grasp of the practicing physician. I hope that this paper may at least partially clarify this situation. Of the diseases associated with splenomegaly, the commonest only will be considered.

HEMOLYTIC ICTERUS

An examination of blood, made February 10, 1931, gave the following results:

Hemoglobin	8.5 gm. in 100 c.c. (51 per cent)
Erythrocytes	2,300,000 in 1 c.mm. of blood
Color index	1.1
Leucocytes	7,400 in 1 c.mm. of blood
Lymphocytes	27 per cent
Monocytes	3 per cent
Neutrophiles	66.5 per cent
Eosinophiles	2.0 per cent
Myelocytes	1.5 per cent
Normoblasts	5 seen
Reticulated erythrocytes	13.4 per cent
Platelets	134,000 in 1 c.mm. of blood

Comment on blood picture: Moderate to marked microcytosis, cells spherical, evidence of active regeneration.

Marked anemia was present. The color index of 1.1 was higher than that seen in simple secondary anemia, and not so high as that which usually occurs in cases of pernicious anemia. Not infrequently the color index in cases of hemolytic icterus is 0.8 or 0.9. Occasionally, in very severe cases, with extreme anemia, it is greater than 1.0. If repeatedly the color index is 0.8 or 0.9, hemolytic icterus may be suspected from this feature alone.

The number of leucocytes was slightly above normal, whereas the percentage of polymorphonuclear neutrophiles was normal. These features are suggestive of active regeneration of the bone marrow, as is also the presence of an occasional neutrophilic myelocyte.

The percentage of reticulated erythrocytes was very much above normal. This is the most trustworthy sign of active production of erythrocytes. The

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reticulated cells are regarded as young erythrocytes not yet completely saturated with hemoglobin. The reticulation is due to a reaction between a vital stain (brilliant cresyl blue) and the basophilic spongioplasm which has not entirely been replaced by hemoglobin.

The combination, therefore, of a moderately high color index with a normal leucocyte count or slight leucocytosis, a normal or increased percentage of polymorphonuclear cells, and a very high percentage of reticulated erythrocytes, is almost pathognomonic of hemolytic icterus, which is a disease of increased hemolysis accompanied by very active regeneration of blood.

Microcytosis, reported after examination of the blood smears, is a most valuable characteristic from the diagnostic standpoint. A very high percentage of microcytes, 60 or 70 per cent, is almost always seen in cases of hemolytic icterus. The microcytes are usually spherical, and appear as deeply stained cells, well filled with hemoglobin. In addition to microcytes, large, oval macrocytes are also seen; their presence, however, is not essential to the diagnosis. The blood smears in cases of hemolytic icterus also show signs of very active regeneration of erythrocytes, especially polychromatophilia and occasional normoblasts. There is a shift to the left of polymorphonuclear leucocytes, that is, a decrease in the number of lobes of the nuclei; possibly 30 per cent or more of the polymorphonuclears may have only two lobes. There may also be evidence of slight immaturity in the granular series, indicating hyperactivity of the bone marrow.

SPLENIC ANEMIA

An examination of blood, made March 19, 1930, gave the following results:

Hemoglobin	5.5 gm. in 100 c.c. (33 per cent)
Erythrocytes	2,225,000 in 1 c.mm. of blood
Color index	0.7+
Leucocytes	2,300 in 1 c.mm. of blood
Lymphocytes	46.5 per cent
Monocytes	1.5 per cent
Neutrophiles	52 per cent
Platelets	124,000 in 1 c.mm. of blood
Reticulated erythrocytes	0.5 per cent

Comment on blood picture: Marked poikilocytosis with hypochromasia, and apparent slight reduction in platelets.

A diagnosis of splenic anemia is made chiefly by exclusion of other diseases associated with splenomegaly. The features of the blood count are only slightly suggestive and by no means constant. Those features which are not infrequently of diagnostic value are exemplified in the blood count which has been presented. There is marked anemia, with a color index of 0.7+. There is definite leucopenia and slight increase in the percentage of lymphocytes, associated with slight decrease of the percentage of neutrophiles. That these alterations in the leucocyte and differential counts do not mean a hypoplastic condition of the bone marrow is evidenced by presence in the blood smear of reticulated erythrocytes and absence of features of inactive regeneration.

In blood smears, on morphologic examination, there not infrequently is marked poikilocytosis, without, however, the marked macrocytosis which is seen

in pernicious anemia; moreover, erythrocytes reveal hypochromasia. The marked poikilocytosis may be regarded as evidence of toxicity, and possibly of abnormal destruction of blood in splenic anemia. Although poikilocytosis is marked only in cases of splenic anemia with severe anemia, it is a more prominent feature than in secondary anemia from hemorrhage.

The diagnosis of splenic anemia sometimes is made in cases in which the leucocyte count is normal or there is even slight leucocytosis. Potential splenic anemia may not be accompanied by reduction in hemoglobin and number of erythrocytes. However, the features which have been considered are sufficiently constant to assist in the diagnosis.

HEMORRHAGIC PURPURA

An examination of blood, made March 24, 1928, gave the following results:

Hemoglobin	27 per cent (Dare)
Erythrocytes	2,060,000 in 1 c.mm. of blood
Color index	0.7–
Leucocytes	3,800 in 1 c.mm. of blood
Lymphocytes	33 per cent
Transitionals	2 per cent
Neutrophiles	62 per cent
Eosinophiles	2 per cent
Basophiles	1 per cent
Reticulated erythrocytes	9.3 per cent
Platelets	80,000 in 1 c.mm. of blood
Bleeding time	30 minutes
Clot retraction	Slight after six hours

Comment on blood picture: Definite evidence of active regeneration, with anisocytosis, polychromatophilia, and many reticulated cells; platelets much reduced in number.

Marked anemia of the secondary type was present. The leucocyte count was slightly below normal, which might in itself suggest reduced activity on the part of the bone marrow; however, in conjunction with a normal percentage of neutrophiles and a high count for reticulated cells, it would not be so interpreted. It should be emphasized, therefore, that a slightly low leucocyte count in the presence of other evidence of active regeneration on the part of the bone marrow cannot be regarded as indicative of hypoplastic marrow. The leucocyte count usually is normal or more than normal in cases of hemorrhagic purpura.

The platelet count was low; the bleeding time was prolonged and the retractility of the clot was delayed. These abnormal features of coagulation are seen chiefly in hemorrhagic purpura, acute aplastic anemia, and the hemorrhagic phase of leucemia. Leucemia was excluded by absence of immature cells. It may be said, in this connection, that not infrequently in my experience immature cells and even stem cells have been mistakenly classified as monocytes and lymphocytes, and care must be exercised not to overlook immaturity. Aplastic anemia was excluded by absence of evidence of hypoplastic marrow; that is, by the high percentage of reticulated cells and normal percentage of polymorphonuclear cells. Consequently, after analysis of such a blood count one would strongly suspect a diagnosis of hemorrhagic (thrombocytopenic) purpura in spite of the presence of slight leucopenia.

Examination of blood films in cases of hemorrhagic purpura reveals the characteristics of active physiologic regeneration in contradistinction to aplastic anemia; in other words, the presence of an appreciable number of reticulated erythrocytes, moderate or marked polychromatophilia, and anisocytosis associated with macrocytosis. Moreover, platelets may not be seen in the films, and usually when platelets are not found the count is below 30,000. If platelets are present they are likely to vary greatly in size and to appear deformed in shape; very large platelets are sometimes present.

ACUTE APLASTIC ANEMIA

An examination of blood, made December 27, 1927, resulted as follows:

Hemoglobin	30 per cent
Erythrocytes	1,540,000 in 1 c.mm. of blood
Color index	0.9+
Leucocytes	2,800 in 1 c.mm. of blood
Lymphocytes	66.5 per cent
Monocytes	7 per cent
Neutrophiles	26.5 per cent
Reticulated erythrocytes	None
Platelets	52,000 in 1 c.mm. of blood
Coagulation time (Lee and White method)	10 minutes
Bleeding time	7 minutes
Clot retraction	Absent in six hours

Comment on blood picture: Morphologic evidence of decreased regeneration with absence of reticulated cells; platelets reduced in number.

This blood count in a case of acute aplastic anemia is presented chiefly because of diagnostic contrast with the preceding count of hemorrhagic purpura. Slight splenomegaly is sometimes present in cases of acute aplastic anemia. In this blood count marked anemia was present; the color index was moderately high. Leucopenia was found, and this was associated with reduction in the percentage of neutrophiles and increase in the percentage of lymphocytes. Leucopenia, with reduction in percentage and in absolute number of neutrophiles, is an indication of reduced activity on the part of the bone marrow. The absence of reticulated cells is also a feature of hypoplastic marrow. The features of coagulation, that is, the prolonged bleeding time and the delayed retractility of clot, together with a low platelet count, are the same as those which might be obtained in a case of hemorrhagic purpura. However, in this count, the definite indications of reduced production of erythrocytes, polymorphonuclear leucocytes, and platelets indicate clearly generalized reduction of activity of bone marrow.

Morphologic study of the blood films in this case also gave evidence of aplastic marrow. The scarcity of reticulated erythrocytes, the absence of polychromatophilia, the low absolute and low relative number of polymorphonuclear neutrophiles, and the presence of old, many lobed polymorphonuclear cells are all characteristics of importance in arriving at the conclusion that regeneration is inactive. In some of the cases a decision with respect to activity of the bone marrow may be very difficult to make and repeated examinations of the films may be necessary before one can determine definitely the degree of regeneration.

INFECTIOUS MONONUCLEOSIS (ACUTE BENIGN LYMPHADENOSIS, BENIGN LYMPHOCYTOSIS, GLANDULAR FEVER)

An examination of blood, made October 20, 1927, revealed the following:

Hemoglobin	61 per cent (Dare)
Erythrocytes	3,840,000 in 1 c.mm. of blood
Leucocytes	14,000 in 1 c.mm. of blood
Lymphocytes	70 per cent
Monocytes	3.5 per cent
Neutrophiles	25.5 per cent
Eosinophiles	0.5 per cent
Basophiles	0.5 per cent

Comment on blood picture: Typical of infectious mononucleosis.

Clinically this disease may simulate leucemia, and a mistake in diagnosis may lead to great chagrin. Slight enlargement of the spleen is the rule; marked splenomegaly is not seen.

In this blood count it will be noted that slight anemia was present, with a low color index. There was definite leucocytosis and a percentage of lymphocytes of 70. In view of these findings, the diagnosis of chronic lymphatic leucemia would immediately suggest itself. However, the percentage of lymphocytes is not as high as that which is usually seen in lymphatic leucemia, and the diagnosis would have to depend on the findings on morphologic examination of the films.

On morphologic examination of blood films the picture was characteristic. The lymphocytes, as a rule, were larger than normal; some of them of leucocytoid appearance; many of them contained vacuoles both in the nucleus and in the cytoplasm, and the nuclei showed some tendency to lobulation. The lymphocytes showed no definite evidence of immaturity. In films of this kind vacuoles may be mistaken for nucleoli, and careful study of the nuclear structure of the lymphocyte is necessary; gradual transition between parachromatin and chromatin is the essential feature in recognition of a mature lymphocyte. It is true that occasionally a slightly immature lymphocyte may be seen in a case of infectious mononucleosis, and sometimes judgment must be suspended. The monocytes are also increased in number, and usually, during the period of recovery, the percentage of monocytes decreases as that of polymorphonuclears increases. The return to a normal blood picture is usually a matter of weeks, but occasionally abnormalities are present for several months.

LYMPHATIC LEUCEMIA WITH NORMAL LEUCOCYTE COUNT

An examination of blood, April 3, 1927, gave the following results:

Hemoglobin	31 per cent
Erythrocytes	2,200,000 in 1 c.mm. of blood
Leucocytes	6,500 in 1 c.mm. of blood
Mature lymphocytes	57.5 per cent
Immature lymphocytes	10.5 per cent
Monocytes	3 per cent
Neutrophiles	29 per cent

Comment on blood picture: Definite immaturity of lymphocytes.

The important feature of this examination of blood obviously is the presence of immaturity of the lymphocytes. Some of them were found to be lymphoblasts or stem cells. The nuclear material of immature lymphocytes is relatively sharply differentiated into chromatin and parachromatin. In spite of a normal leucocyte count, which is not uncommon in acute leucemia, a positive diagnosis can be made from the blood. Slight to marked splenomegaly may be present in cases of lymphatic leucemia. It is unnecessary to present a blood count of chronic lymphatic leucemia with leucocytosis or chronic myelogenous leucemia with leucocytosis. The diagnosis in these conditions is readily made. In the acute and subacute forms of leucemia, 30 per cent or more of the cells may be myeloblasts, or stem cells. In chronic types a small proportion of cells may be slightly immature, and an occasional stem cell may be found; in periods of acute exacerbation of chronic leucemia, extensive immaturity and hemorrhagic features may be present.

ACUTE MONOCYTIC LEUCEMIA WITH HEMORRHAGIC FEATURES

An examination of the blood, made October 21, 1931, revealed the following:

Hemoglobin	4.7 gm. (28 per cent)
Erythrocytes	1,400,000 in 1 c.mm. of blood
Leucocytes	6,600 in 1 c.mm. of blood
Lymphocytes	14.0 per cent
Monocytes	11.0 per cent
Immature monocytes	36.0 per cent
Neutrophiles	24.0 per cent
Eosinophiles	5.0 per cent
Basophiles	3.0 per cent
Promyelocytes	2.0 per cent
Leucoblasts	2.0 per cent
Stem cells	3.0 per cent
Normoblasts	13 seen
Platelets	70,000 in 1 c.mm. of blood
Reticulated erythrocytes	2.6 per cent
Bleeding time	24 minutes
Clot retraction	Absent in 5 hours
Coagulation time of venous blood	5 minutes

Comment on blood picture: Definite monocytosis, with many immature monocytes, including even stem cells. Also, immaturity in the myeloid line, with intermediate forms present, which might also be traced to the stem cell. Myeloid cells probably due to secondary stimulation.

Monocytic leucemia is a third form of leucemia which has come under discussion especially in the last few years. The diagnosis depends almost entirely on careful study of blood smears. This blood count not only emphasizes the importance of study of the morphology of the blood in connection with the diagnosis of acute leucemia, but also demonstrates a case in which the leucocyte count is normal and in which hemorrhagic features are marked. Results of the various coagulation tests are identical with those of acute aplastic anemia in the hemorrhagic phase, and of thrombocytopenic purpura; similar changes in coagulation are seen in arsenobenzol purpura, in acute exacerbations of chronic leuce-

mia, and following excessive roentgen therapy; diagnostic distinction from acute leucemia, then, depends on the morphologic characteristics of the cells.

AGRANULOCYTIC ANGINA

An examination of blood, April 2, 1928, revealed the following:

Hemoglobin	55 per cent (Dare)
Erythrocytes	3,650,000 in 1 c.mm. of blood
Color index	0.7+
Leucocytes	1,100 in 1 c.mm. of blood
Lymphocytes	92.5 per cent
Monocytes	2.0 per cent
Neutrophiles	4.5 per cent
Basophiles	1.0 per cent

These results give evidence of moderate anemia; it should be noted that the anemia is not extreme. The outstanding feature, however, is the leucopenia, associated with a very much reduced absolute number of neutrophiles, and a decreased relative percentage of neutrophiles. The percentage of lymphocytes is high, but the absolute number of lymphocytes is reduced and immature lymphocytes are not present. The absence of severe anemia is evidence against the diagnosis of acute aplastic anemia. On careful analysis, therefore, this blood count alone is very suggestive of agranulocytosis.

In morphologic study of the films in cases of agranulocytosis and hypogranulocytosis, few changes from the normal morphologic structure of the lymphocytes, monocytes, and neutrophiles are, as a rule, to be noted. However, the neutrophiles are frequently "toxic" in appearance, with heavy granulation and distorted nuclei; neutrophiles are not infrequently absent.

HODGKIN'S DISEASE

An examination of blood, made October 9, 1931, disclosed the following:

Hemoglobin	10.5 gm. (63 per cent)
Erythrocytes	4,760,000 in 1 c.mm. of blood
Leucocytes	4,000 in 1 c.mm. of blood
Lymphocytes	18.0 per cent
Monocytes	7.0 per cent
Neutrophiles	69.5 per cent
Eosinophiles	5.5 per cent
Basophiles	1.0 per cent
Reticulated erythrocytes	0.8 per cent

Comment on blood picture: Definite monocytosis, with a shift to the right, toxic neutrophiles with a shift to the left, and moderate eosinophilia (see last paragraph in section headed "The right and left shift"). These features usually indicate Hodgkin's disease.

Very rarely, Hodgkin's disease may be present without other recognizable clinical manifestations than splenomegaly. Usually the diagnosis in such a case is made only at operation or at necropsy. However, there are certain features in the blood which when present make one at least suspicious of the existence of Hodgkin's disease; these features are monocytosis and eosinophilia.

Morphologically, in this case, eosinophilia was noted in the blood picture, together with monocytosis and a shift of the monocytes to the right; the shift to the right manifests itself by indentation of nuclei. The polymorphonuclear

neutrophiles showed marked evidence of toxicity; in other words, the granulations were coarse, the nuclei deformed, and in the nuclear material there was poor differentiation between chromatin and parachromatin.

POLYCYTHEMIA VERA

An examination of the blood, made July 10, 1931, revealed the following:

Hemoglobin	19.9 gm. in 100 c.c. (119 per cent)
Erythrocytes	7,300,000 in 1 c.mm. of blood
Leucocytes	10,800 in 1 c.mm. of blood
Lymphocytes	11.0 per cent
Monocytes	4.0 per cent
Neutrophiles	83.0 per cent
Eosinophiles	1.0 per cent
Basophiles	1.0 per cent
Normoblasts	1 seen
Reticulated erythrocytes	1.1 per cent
Viscosity	14.8 times that of water
Blood volume	7,229 c.c. total volume (129 c.c. for each kilogram of body weight)

Comment on blood picture: Marked piling of erythrocytes and fragility of leucocytes.

The blood of polycythemia is considered, especially because the blood count may vary considerably. In fact, the erythrocyte count may be normal or even less than normal, especially if gastrointestinal hemorrhage has recently occurred. In a case of splenomegaly associated with a normal blood count or moderate anemia, a mistaken diagnosis of splenic anemia may be made. In a case of this type, the former history, the former blood counts, and estimations of viscosity of the blood and of blood volume may be necessary to arrive at a correct diagnosis.

In the results presented, the value for hemoglobin is high, and the erythrocyte count is high, with moderate leucocytosis; the number of polymorphonuclear cells is increased, both relatively and absolutely. These features would indicate either extremely active bone marrow, or very much decreased destruction of blood. Normal viscosity, compared with water, is 4.5, and the increased viscosity of 14.8 would be suggestive of polycythemia. Normal blood volume is approximately 90 c.c. for each kilogram of body weight, and the increased blood volume of 129 c.c. for each kilogram is probably the most important finding with respect to diagnosis.

The existence of relative or secondary polycythemia must also be considered in a case which presents this blood count. Usually a history of asthma, chronic pulmonary fibrosis, or some other evidence of pulmonary obstruction, or of cardiac disease, may be elicited in cases of relative polycythemia, showing slight increase in the erythrocyte count, and slight increase in blood volume.

Morphologically, there is little abnormal in the blood smears. The erythrocytes, on account of their increased number, usually are abnormally "piled up" but if smears are made very thin, this characteristic may not be evident.

SPLENOMEGALY OF INFANCY AND CHILDHOOD

These conditions may conform to well recognized syndromes, such as those of splenic anemia, hemolytic icterus, hemorrhagic purpura, and syphilitic spleno-

megaly. There is, however, a large group of cases in which a very peculiar type of blood picture may result from infections, deficient dietary regimen, nutritional disorders, and metabolic diseases. It must be remembered that the blood of infants reacts differently from that of adults, and rather readily reverts to the fetal type.

In the syndrome known as von Jaksch's disease, there is anemia with low color index, leucocytosis usually with relative and absolute lymphocytosis, small numbers of myelocytes and metamyelocytes in the peripheral blood, and, in some cases, a large number of immature erythrocytes. None of these features, however, is constant, and the syndrome may be a secondary manifestation. In the anemia of infancy, immature cells are very frequently present in the blood smears, and too much stress cannot be laid on this finding; one must guard against a too hasty diagnosis of leucemia. Probably the most important consideration in studying blood smears of infancy is to decide on the evidence for or against active regeneration on the part of the bone marrow, and it must be constantly kept in mind that many of the morphologic features may be explained by assuming a reversion to the fetal type of production of blood.

MISCELLANEOUS DISEASES

Pernicious anemia occasionally is accompanied by moderate splenomegaly. The spleen is palpable at some time during the course of the disease in most of the cases. The features of the blood count in pernicious anemia are so generally familiar that it is unnecessary to comment on them. Morphologic characteristics of the blood film are macrocytosis, with a fair proportion of oval macrocytes, and a shift to the right of polymorphonuclear leucocytes; that is, a higher proportion than normal of many lobed nuclei, connected by fine strands. It may be emphasized that poikilocytosis, ordinarily described as a constant feature, although frequently present, may be almost entirely absent.

Eosinophilic hyperleucocytosis with splenomegaly is a rare clinical syndrome. In this condition the eosinophilic polymorphonuclear leucocytes may be increased in number, both relatively and absolutely, to an extreme degree. The leucocyte count is not infrequently high, and it has been suspected that cases of this type may in reality represent a peculiar form of leucemia; however, it is much more likely that the eosinophilia is an unusual reaction to chronic recurring infection.

Lymphosarcoma, it has frequently been thought, may be suspected in the presence of suggestive clinical findings when the differential count discloses relative and absolute increase of lymphocytes, with a normal leucocyte count or slight leucocytosis. This suspicion is sometimes corroborated on pathologic examination of the spleen after splenectomy, but just as frequently there is no evidence of lymphocytic hyperplasia in the spleen when the blood count has revealed the features mentioned.

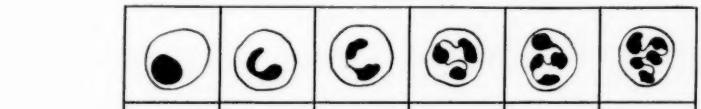
Sickle cell anemia is a very interesting syndrome, and is essentially a form of hemolytic anemia. Sickle cells are frequently found in the blood of negroes without anemia. The erythrocytes assume the sickle-shaped contour in fresh wet preparations. In sickle cell anemia the spleen may be considerably enlarged and the clinical syndrome may be suggestive of hemolytic icterus. Fragility of the erythrocytes, however, is not increased. The spleen not infrequently becomes atrophied later in the disease. Morphologically, aside from the sickle-

shaped erythrocytes, leucocytosis is usually present, and a few immature leucocytes may be found.

Abscess of the spleen has, in some instances, been accompanied by an extreme degree of polymorphonuclear leucocytosis. Leucocytes may number as high as 50,000 in each cubic millimeter of blood.

Marble bone disease, an affection in which there is increased density of the bones, with encroachment on the marrow cavity, usually reveals a blood count that is slightly suggestive of myelogenous leucemia. In the later phases of the disease the leucemic type of blood picture may become more marked.

Subacute bacterial endocarditis occasionally causes the appearance of large reticular cells in the blood smears made from the ear.



Normal		10	25	47	16	2
Right		9	23	34	24	10
Left	5	30	37	20	8	

Fig. 1.—Lobation of nucleus of the neutrophile with percentage of cells found in the normal, in a left shift and in a right shift. Taken from Watkins, C. H., and Heck, F. J.: The Practical Value of Examination of Blood Smears, Minnesota Med. 13: 860-864, 1930. (Their normal values were taken from Cooke, W. E.: Further Observations on the Macropholycyte. Brit. M. J. 1: 800-804, 1929.)

THE RIGHT AND LEFT SHIFT

In view of the consideration of the right and left shift of the neutrophile in the various diseases, Fig. 1 is presented. It will be seen that in normal counts approximately half the cells have nuclei of three lobes, whereas with a shift either to the right or to the left, one-third or less of the cells will have nuclei of three lobes. With a shift to the right, a high percentage of polymorphonuclear cells will have five lobes or more. Occasionally a cell is seen containing eight or nine lobes. With a shift to the left, two-thirds of the cells may have either crescentic nuclei or two lobes. Many lobed nuclei are most frequently seen in the blood of patients with pernicious anemia, and sometimes this feature may be observed even when the blood count is normal; in pernicious anemia with nuclei of many lobes, the lobes frequently are narrow and smooth in contour, and the interlobar strands are elongated. In severe infections, the shift to the left may be extreme, metamyelocytes may be numerous and even myelocytes may be present.

A definite shift is also frequently noted in the maturity of the monocyte. Normally about half the monocytes present indented nuclei. With a shift to the right a majority of the cells will have indented nuclei. With a shift to the left a majority of the nuclei are circular or oval in appearance, and may contain heavy, "toxic" granulations.

SUMMARY

A series of blood counts, differential counts, and results of the morphologic examination of blood pictures has been presented in diseases with slight, moderate, or marked splenomegaly with the purpose of demonstrating the various features of the blood which frequently are of value in diagnosis. Careful analysis of the characteristics of the anemia, the features of the differential count, the presence of immature cells, the percentage of reticulated erythrocytes, and other evidences of active and inactive regeneration of blood, and the characteristics of the cells on study of the blood film, not infrequently lead to important inferences with respect not only to the diagnosis but also to the prognosis, and to more accurate estimation of the probable results of various methods of treatment. My plea is for more careful analysis of a very common laboratory examination, the results of which too frequently are considered in a superficial manner. The physician can easily become accustomed to examination of blood smears personally, with immediate profit to himself and ultimate benefit to the patient. Many blood pictures are quickly recognized; others, however, require repeated examinations of the blood film before one can be reasonably satisfied.

THE BLOOD PICTURE IN HYPERTHYROIDISM AND IN HYPOTHYROIDISM*

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THE report presented here is based on a study of 1200 routine blood counts made in consecutive cases of hyperthryroidism. Differential counts were made in 250 of these cases.

The report includes routine counts made in 17 cases in which death followed uncomplicated hyperthyroidism and in 51 cases in which postoperative hypothyroidism occurred.

In addition a number of smaller groups are reported in which blood counts were made by one of us (J. H. D.) in a more careful and more detailed manner. These groups include: (1) twenty unselected cases of hyperthyroidism, (2) twelve cases in which counts were made before and after thyroidectomy, (3) twenty cases of postoperative hypothyroidism, and (4) ten cases of hyperthyroidism and ten cases of hypothyroidism in which filament, nonfilament counts were made.

REVIEW OF THE LITERATURE

The literature regarding the blood findings in thyroid disorders shows a marked variability in the results and in the conclusions drawn from them. For many years investigators have sought to establish a definite and constant picture whereby they could be aided in the diagnosis and prognosis in cases of hyperthyroidism and hypothyroidism. In 1885, Horsley[1] stated that "marked anemia followed the loss of the thyroid gland." Since that time findings have been reported that run the gamut from marked anemia to no anemia, from lymphocytosis, to no lymphocytosis, and from leucopenia to leucocytosis. The conclusions drawn from these varied findings have shown a similar variation; consequently it is difficult for one to reach

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any definite conclusions from the literature, and it was this fact which prompted us to make the investigation here reported.

Some writers have reported normal blood findings in hypothyroidism[3, 4, 5] while others report a mild anemia[6, 7, 8, 9, 10, 24, 39] and still others report red cell counts varying from 3,000,000 to 4,500,000[11, 12, 13, 14, 15, 16] and lower.[17, 18] Falta[20] and others[12, 21] mention hemoglobin estimations below 60 per cent in cases of hypothyroidism and Howard[22] reports cases with a hemoglobin of 75 per cent.

Certain writers have mentioned that in hypothyroidism leucocytosis is present.[2, 12, 21] Normal white cell counts[16, 22] and leucopenia[7, 25, 26, 27] are also reported. Schermann[38] reports that in rabbits a marked reduction in the number of white blood cells follows thyroidectomy.

Kocher[27] found the eosinophile count to be normal in hypothyroidism, but a few believe it to be increased.[7, 20, 26]

Emery[19, 23, 28] states that in myxedema the blood picture is not constant and mentions reduced hemoglobin, a normal white cell count, a decrease in the polymorphonuclear count and perhaps relative or absolute lymphocytosis as frequent findings.

Plummer[45] reported the blood counts in a much larger group of cases of hyperthyroidism than had been reported before and showed that there was a tendency to lymphocytosis, that a relatively low polymorphonuclear count was a large factor in the production of this change and that the degree of lymphocytosis was a poor index as to the degree or duration of the disease.

The statements regarding the blood findings in hyperthyroidism are also quite varied. Many writers believe that there is a characteristic blood picture in Basedow's disease[27, 30, 31, 36, 41], which is considered of definite diagnostic importance. Some have declared that the lymphocytosis in hyperthyroidism parallels the severity of the disease.[40] Roth[37] believes that the reduced hemoglobin, leucopenia, and lymphocytosis in hyperthyroidism is of prognostic as well as diagnostic value. Others disagree with these views and state that there is no characteristic blood picture in hyperthyroidism[29, 32, 33, 34, 35] and that the lymphocyte count is not in proportion to the severity of the disease.[42]

RESULTS OF BLOOD COUNTS

The average counts for the whole group of 1200 routine blood counts made in 1200 consecutive cases of hyperthyroidism were as follows:

Hemoglobin	82.5 per cent
Erythrocytes	4,555,154
Leucocytes	7,034

For the 250 cases in which differential counts were done the results were as follows:

Leucocytes	7,954.7
Polymorphonuclears (relative)	62.21
Polymorphonuclears (absolute)	4,948.7
Eosinophiles (relative)	0.49
Eosinophiles (absolute)	39.4
Basophiles (relative)	0.09
Basophiles (absolute)	7.5
Lymphocytes (relative)	30.3
Lymphocytes (absolute)	2,412.3
Monocytes (relative)	5.7
Monocytes (absolute)	460.5
Transitionals (relative)	0.90
Transitionals (absolute)	75.5

From the above results it is seen that the red and white cell counts are normal. There is an absolute and relative lymphocytosis both of large and small cells. There is a relative decrease and a slight absolute decrease in the number of polymorphonuclear neutrophiles and to a less degree in the number of eosinophiles or basophiles. There is a slight reduction of hemoglobin.

In order to study the relationship between the polymorphonuclear and lymphocyte counts two smaller groups were arranged. The 50 counts in which the number of leucocytes was greatest were grouped together. The average count was as follows:

Leucocytes	11,974
Polymorphonuclears (relative)	68.7
Polymorphonuclears (absolute)	8,232.4
Lymphocytes (relative)	26.0
Lymphocytes (absolute)	3,113.8
Monocytes (relative)	4.5
Monocytes (absolute)	539.8

If 5000 is considered an approximate average neutrophile count, it will be seen that the polymorphonuclears have increased in number by more than 3000 cells. On the other hand, the lymphocyte count the normal average of which is in the neighborhood of 1500, has been increased by only slightly over 1600 cells. In this group, then, the absolute lymphocyte count is actually greater than the average for the total series. The increase in the number of polymorphonuclears, however, is so great that the relative lymphocytosis becomes less than the average lymphocytosis for the whole group.

Compare the above figures with those of the averages for a group composed of the 50 lowest white cell counts.

Leucocytes	4,985
Polymorphonuclears (relative)	57.8
Polymorphonuclears (absolute)	2,861.3
Lymphocytes (relative)	36.4
Lymphocytes (absolute)	1,817.5
Monocytes (relative)	4.7
Monocytes (absolute)	234.8

In this group the facts pointed out above are emphasized. The number of polymorphonuclears has fallen to more than 2000 below the normal average, while the absolute number of lymphocytes is still maintained at a figure definitely above the normal. Obviously then in hyperthyroidism the blood counts in cases in which leucopenia is present will demonstrate a greater lymphocytosis than in cases in which no leucopenia is present. This fact is accounted for by the relatively greater fluctuation of the polymorphonuclear cells.

Since lymphocytosis has been shown to be more common when hyperthyroidism is present than in the normal individual, it must have some diagnostic value. The frequency with which lymphocytosis may be present in hyperthyroidism is indicated in Table I representing a total of 266 cases.

TABLE I

RELATIVE LYMPHOCYTE COUNT	NUMBER OF CASES	RELATIVE NUMBER OF CASES
PER CENT		PER CENT
0-15	12	4.5
15-19	13	4.8
20-24	33	12.4
25-29	48	18.0
30-39	84	31.5
40 and above	76	28.5

In 78 per cent of 266 cases the relative lymphocyte count was 25 per cent or above. In 60 per cent of the cases it was 30 per cent or above.

Let us now examine a few groups of cases in which the average height of the basal metabolic rate varies greatly in order to determine whether or not the degree of lymphocytosis is directly related to the severity of the disease.

The first group comprises 36 cases of mild hyperthyroidism. The basal metabolic rate in these cases varied between plus 18 per cent and plus 30 per cent. This group does not include those cases in which the metabolism was below plus 18 per cent, because it was felt that below this level there was a greater possibility of error in the diagnosis. It was thought that a metabolic rate of not more than 30 per cent would be the best index in selecting mild cases. The duration of the disease was not considered. The clinical diagnosis was that of hyperthyroidism in each instance. The blood was not taken during fasting but was taken in all cases within twenty-four hours of the basal metabolism estimation.

Leucocytes	8574
Polymorphonuclears (relative)	68.1
Polymorphonuclears (absolute)	5836
Lymphocytes (relative)	26.3
Lymphocytes (absolute)	2257
Monocytes (relative)	4.6
Monocytes (absolute)	398
Transitionals (relative)	0.9
Transitionals (absolute)	88

Here we see a total white count which is somewhat above the average. The absolute number of lymphocytes is raised more as compared with the polymorphonuclears. There is, however, no lymphocytosis.

The next group is composed of 36 cases of moderate and severe hyperthyroidism. The basal metabolic rate in these cases was plus 40 per cent and above. No cases with any outstanding infection or complication were included. The blood counts were not made on fasting blood and the blood was taken on the day preceding the basal metabolism estimation.

Leucocytes	7207
Polymorphonuclears (relative)	62.1
Polymorphonuclears (absolute)	4478.6
Lymphocytes (relative)	31.5
Lymphocytes (absolute)	2271
Monocytes (relative)	5.3
Monocytes (absolute)	386
Transitionals (relative)	0.9
Transitionals (absolute)	71

On comparing the findings in these severe cases with those in the previous group, we see that the absolute lymphocyte count is very nearly the same as that found in the mild cases. In this group, however, there is a much lower polymorphonuclear count, so that there is a lower total leucocyte count and a mild relative lymphocytosis.

The third group is composed of 17 cases in which death resulted from hyperthyroidism before operative interference was attempted. This group includes no cases in which a complication was a contributing factor, except in the case of those changes which appear immediately preceding death. The blood counts were made from one to seven days before death.

Leucocytes	9391
Polymorphonuclears (relative)	73.0
Polymorphonuclears (absolute)	6861.8
Lymphocytes (relative)	19.4
Lymphocytes (absolute)	1828.3
Monocytes (relative)	6.8
Monocytes (absolute)	649.2
Transitionals (relative)	0.4
Transitionals (absolute)	45.4

Here again the low relative lymphocyte value is not so much due to any great change in the absolute number of lymphocytes as to the marked increase in the number of polymorphonuclears.

The fourth group comprises cases of postoperative hypothyroidism. Fifty-one cases are included in which the basal metabolic rate varied between minus 15 per cent and minus 30 per cent, the average rate being minus 23.2 per cent. The blood counts in these cases were made on fasting blood on the morning of the metabolism test.

Leucocytes	8333
Polymorphonuclears (relative)	62.0
Polymorphonuclears (absolute)	5168.6
Lymphocytes (relative)	29.9
Lymphocytes (absolute)	2495.1
Monocytes (relative)	6.9
Monocytes (absolute)	575.4
Transitionals (relative)	0.2
Transitionals (absolute)	21.6

The same comment may be made here as in the case of the previous groups. It appears to be obvious, then, that the relative lymphocytosis bears a very close relationship to the total white count and especially to the absolute number of polymorphonuclears present. It bears little or no relationship to the severity of the disease.

Let us now examine a group of twenty-eight cases, which was arranged in an attempt to determine whether or not the relative lymphocyte count bore any definite relationship to the degrees of hyperthyroidism in individual cases. Cases were selected in which after one night's bed rest in the hospital the basal metabolic rate was in excess of plus 40 per cent.

In these cases a blood count was made on fasting blood immediately following the metabolism test. Five days after thyroidectomy, if the wound had healed per primam, the basal metabolism test and blood count were repeated as before. The results are summarized in Table II which represents the average heights of metabolism and average blood counts on admission and five days postoperatively.

The relative number of lymphocytes showed an average decrease postoperatively, but there was a very slight decrease in the absolute number. There was a definite rise in the absolute and relative number of neutrophiles.

TABLE II

	PREOPERATIVELY	POSTOPERATIVELY
Basal Metabolism	56.4	10.9
Erythrocytes	4,540,000	4,408,000
Leucocytes	6,730	7,460
Polymorphonuclears (relative)	63.2	70.2
Polymorphonuclears (absolute)	4,253	5,236
Lymphocytes (relative)	33.4	26.0
Lymphocytes (absolute)	2,247	2,039

TABLE III

BLOOD COUNTS IN TWENTY CASES OF HYPERTHYROIDISM

NO.	BASAL METABOLISM	PACKED CELLS PER CENT OF NORMAL	RED BLOOD CELL COUNT	HEMOGLOBIN	VOLUME INDEX	COLOR INDEX	SATURATION INDEX	INITIAL WHITE BLOOD CELL COUNT	INITIAL DIFFERENTIAL SMEAR				
									POLYMORPHONUCLEARS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	BASOPHILES
1	+45	91	4,810,000	84	0.94	0.87	0.92	6,400	59	31	4.5	2.5	1
2	+ 9	95	4,000,000	80	1.1	1.0	0.84	7,350	43	48	6	3	0
3	+39	97	4,870,000	78	0.88	0.70	0.80	5,350	60.5	35	2.5	2	0
4	+53	73	4,280,000	78	0.85	0.91	1.0	5,800	68	30	1.5	0.5	0
5	+45	84	4,700,000	78	0.89	0.82	0.92	4,700	37	58.5	2.5	2	0
6	+44	93	5,370,000	84	0.93	0.78	0.9	4,800	32	55	10	3	0
7	+36	73	3,850,000	71	0.94	0.92	0.97	6,500	51	46.5	2	1.5	0
8	+64	75	3,040,000	68	1.2	1.1	0.9	4,900	59	35	3	3	0
9	+49	91	4,970,000	84	0.91	0.84	0.92	3,850	47	44	7	2	0
10	+49	82	4,820,000	78	0.85	0.81	0.95	4,850	54	33	7	6	0
11	+65	93	5,190,000	91	0.9	0.88	0.99	8,300	53.5	32	7	7.5	0
12	+24	93	4,660,000	84	1.0	0.9	0.9	6,600	63	24	10	3	0
13	+62	84	4,510,000	84	1.0	0.93	0.92	6,500	37.5	46.5	11	3.5	1.5
14	+24	71	2,880,000	65	1.2	1.1	0.91	4,110	51	38.5	7	3.5	0
15	+ 3	95	4,940,000	80	0.96	0.81	0.83	3,700	58.5	30	10	1.5	0
16	+71	91	4,990,000	80	0.91	0.80	0.87	5,450	48	40	10	2	0
17	+64	82	5,120,000	78	0.82	0.78	0.95	12,450	68.5	23	6.5	1	1
18	+46	66	3,860,000	58	0.85	0.75	0.87	6,350	56	34	8.5	1.5	0
19	+34	73	4,360,000	65	0.87	0.74	0.89	7,100	39.5	47	9.5	4	0
20	+ 9	88	4,730,000	78	0.93	0.82	0.88	7,500	41.5	46.5	8.5	3	0.5

Case 2.—Patient has hyperthyroidism clinically; exophthalmos and all external eye signs, loss of weight; history of plus 35 basal metabolism rate a short time before coming to the Clinic.

Case 15.—Hyperthyroidism clinically. Patient had two x-ray treatments four weeks before admission.

Case 20.—Hyperthyroidism clinically. Patient has had no iodine treatment.

The relative number of lymphocytes was decreased postoperatively in 22, 78.5 per cent, of the 28 cases. Thus the relative number of lymphocytes may serve as some guide to improvement in single cases.

Since the foregoing data have all been based on blood counts made in a routine manner, it was felt that it would be interesting to check these results against a much smaller series made in a more painstaking way by the same individual each time.

The following data are based on such counts made by one of us (J. H. D.). The first group is composed of 20 unselected cases of hyperthyroidism. The counts were all made at about the same time of day and with the same instruments each time. An analysis of the findings in this group gave the following averages:

The average basal metabolism was plus 42.7 per cent.

Leucocytes	6,710
Lymphocytes (relative)	38.5
Lymphocytes (absolute)	2,343
Polymorphonuclears (relative)	51.2
Polymorphonuclears (absolute)	3,435
Erythrocytes	4,497,000
Hemoglobin (Haden-Hausser instrument)	77.3
Volume index	0.94
Color index	0.86
Saturation index	0.9

In 55 per cent of the cases the hemoglobin was below 80 per cent.

In seven cases the volume index was below 0.9; in three cases it was above 1.

In 13 cases, 65 per cent, the color index was below 0.9; in two cases it was below 0.8; and in two cases it was above 1.

In seven cases, 35 per cent, the saturation index was below 0.9.

A consideration of the initial differential counts in this group shows that in 18 or 90 per cent a relative lymphocytosis of 30 per cent or above was present, and that in 45 per cent a relative lymphocytosis of above 40 per cent was present. Of the latter group, in seven there was an absolute increase in the number of lymphocytes as compared with the normal count and as compared with the average absolute count of the series (2343). Of these seven cases in which a relative lymphocytosis of 40 per cent or above was present in each there was an absolute decrease in the number of polymorphonuclear cells.

In 80 per cent of this series the relative polymorphonuclear count was below 60 per cent. The average absolute polymorphonuclear count was 3435.5 which is 1334 less than the accepted average normal count.

The monocyte, eosinophile, and basophile counts were essentially normal (Table III).

In twelve cases of hyperthyroidism blood counts were made on the admission of the patient to the hospital, and repeated from four days to one and one-half months postoperatively. In all cases the basal metabolism had become greatly reduced between the time of the first and the last blood counts. In six cases there was an increase and in six there was a decrease in the absolute number of lymphocytes. The relative lymphocytosis was reduced in every case, however, obviously because of a comparable rise in the polymorphonuclear count (Table IV).

TABLE IV

RELATIVE AND ABSOLUTE LYMPHOCYTOSIS IN TWELVE CASES OF HYPERTHYROIDISM

NO.	BASAL METABOLIC RATE	PREOPERATIVE RELATIVE LYMPHOCYTE COUNT	POSTOPERATIVE RELATIVE LYMPHOCYTE COUNT	PREOPERATIVE ABSOLUTE LYMPHOCYTE COUNT	POSTOPERATIVE ABSOLUTE LYMPHOCYTE COUNT	
						DAYS POSTOPERATIVE
1	+34	47%	34%	3337	3434	(16 d.)
2	+49	44%	40%	1694	1880	(1½ mo.)
3	+53	30%	21%	1740	2845	(7 d.)
4	+64	35%	28.5%	1715	3947	(4 d.)
5	+45	58.5%	32%	2749	3200	(4 d.)
6	+44	55%	42%	2640	3297	(27 d.)
7	+ 9	48%	24%	3528	1599	(5 d.)
8	+36	45.5%	21%	2993	1921	(8 d.)
9	+ 9	46.5%	27.5%	3487	2415	(8 d.)
10	+46	34%	16.5%	2159	2079	(14 d.)
11	+39	35%	23%	1872	1495	(3 d.)
12	+65	32%	24.5%	2656	2621	(4 d.)

In twenty cases of postoperative hypothyroidism blood counts were made in the same careful manner. The average basal metabolism in 19 cases of this series was minus 23.1 per cent (see Table V). The average counts were as follows:

Leucocytes	6905
Lymphocytes (relative)	36.5
Lymphocytes (absolute)	2453.5
Polymorphonuclears (relative)	56.2
Polymorphonuclears (absolute)	3929.5
Erythrocytes	4,611,700
Volume index	0.95
Color index	0.87
Saturation index	0.88
Eosinophiles, monocytes and basophiles	Normal in all cases
Hemoglobin	80.0

In this group in only three cases was the erythrocyte count below 4,000,000. The hemoglobin average for the series would have been somewhat lower had it not been for one case in which there was a slight polycythemia of 5,690,000 with a hemoglobin of 104 per cent. In 50 per cent of the counts in cases of hypothyroidism the hemoglobin was below 80 per cent.

In this group the average color index, volume index, and saturation index were all slightly below normal as was noted also in the group of cases of hyperthyroidism. In five cases the volume index was below 0.9 and in four it was above 1 (Table V).

In twelve cases the color index was below 0.9.

A filament, nonfilament count was made in ten cases of hypothyroidism and the average number of nonfilamented cells was found to be 19.3. In each of these cases the total leucocyte count was normal. In ten cases of hyperthyroidism the average preoperative nonfilament count was 12.3. In these same cases the average postoperative nonfilament count was 12.1.

In one instance the total white cell count was raised to 13,850 postoperatively and the nonfilament count was 25; in the other cases the white cell counts were all within normal limits.

Comparing the counts obtained in the smaller group with the routine counts,

TABLE V

BLOOD COUNTS IN TWENTY CASES OF HYPOTHYROIDISM

NO.	BASAL METABOLISM	PACKED CELLS PER CENT OF NORMAL	HEMOGLOBIN	VOLUME INDEX	COLOR INDEX	SATURATION INDEX	RED BLOOD CELL COUNT	WHITE BLOOD CELL COUNT	POLYMORPHONUCLEARS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	BASOPHILES
1	−28	82	71	1.1	1.0	0.86	3,565,000	3,700	60	35	3	2	
2	−37	88	80	1.0	0.94	0.9	4,280,000	4,850	43	49	6	2	
3	−24	97	84	1.01	0.87	0.86	4,820,000	7,050	41	49	8	2	
4	−25	100	84	1.0	0.84	0.84	5,050,000	5,150	60.5	30	7.5	1	1
5	−16	97	91	1.01	0.94	0.93	4,810,000	9,800	53	34	10	2	1
6	+ 3	84	75	0.84	0.75	0.88	5,000,000	5,650	51	46	2	1	
7	−37	100	91	1.0	0.93	0.91	4,850,000	8,500	62	30	6	2	
8	−14	100	91	0.9	0.82	0.91	5,560,000	8,200	69	27	1	3	
9	−12	95	84	0.96	0.87	0.88	4,840,000	5,100	53.5	38	5	3	0.5
10	− 9	73	58	0.82	0.65	0.69	4,440,000	13,050	63	30	4.5	2	0.5
11	−30	95	91	0.98	0.94	0.95	4,830,000	8,800	59.5	31	5.5	4	
12	−28	68	71	0.75	0.78	1.0	3,400,000	4,500	62	34.5	0	2.5	1
13	−27	91	78	0.96	0.82	0.85	4,720,000	8,650	56.5	38	1.5	2.5	1.5
14	−31	86	78	1.0	0.95	0.90	4,130,000	6,500	61	33.5	4	1.5	
15	−23	73	65	0.91	0.81	0.89	4,010,000	6,650	61	33	4.5	1.5	
16	−30	88	78	0.88	0.78	0.88	5,160,000	7,400	59	33	5.5	0.5	2
17	−17	84	84	0.85	0.85	1.0	4,910,000	5,550	41.5	51	3	4.5	
18	−19	88	71	1.2	1.0	0.80	3,600,000	6,000	49	44.5	4.5	2	
19	− 4	84	78	1.0	0.96	0.92	4,570,000	7,250	60.5	32	5	1.5	1
20	−28	115	104	1.01	0.91	0.9	5,690,000	5,750	59	32.5	6.5	2	

Case 6.—Patient had basal metabolism rate of minus 25 last year and has had thyroid extract intermittently since. Clinically she has hypothyroidism. This case is not included in average basal metabolism rate for group.

Case 10.—Basal metabolism rate has been minus 22. Patient has had thyroid extract at intervals for three years. Clinically she now has hypothyroidism, marked improvement on thyroid extract.

Case 19.—Patient has definite symptoms of hypothyroidism even though basal metabolism rate is only minus 4. Previously she has had marked hypothyroidism with all signs and has improved on thyroid extract.

the findings are approximately the same except that in the former series of cases the total leucocyte count is slightly lower, the relative lymphocyte count is a trifle higher, and the relative polymorphonuclear count is slightly lower.

CONCLUSIONS

1. In hyperthyroidism, the red blood cell count is normal but there is a slight reduction of hemoglobin.

2. There is a relative lymphocytosis of 30 per cent or above in 60 per cent of the large series of cases of hyperthyroidism and in 90 per cent in the smaller series. The presence of a lymphocytosis is therefore of some diagnostic value.

3. The relative lymphocytosis is dependent on two factors in the blood: (a) the absolute lymphocyte rise, (b) the total leucocyte count.

The total leucocyte count has been found to be variable, the variation being due, chiefly, to the variation in the neutrophiles. There is a greater lymphocytosis, therefore, in the presence of leucopenia.

4. In 28 cases examined while the metabolic rate was high, and again after operation, the average lymphocyte count was found to be lowered by operation. In the majority of cases there was a fall in the relative number of lymphocytes present. In the series of 20 cases counted apart from the routine counts 50 per cent of the cases showed an absolute increase of lymphocytes postoperatively (counted from four days to one and one-half months postoperatively), and 50 per cent showed an absolute decrease. All showed a relative decrease.

5. The degree of lymphocytosis on one estimation is not an index of the severity of the hyperthyroidism.

6. The hemoglobin in hypothyroidism is reduced slightly as in hyperthyroidism. The degree of relative lymphocytosis in hypothyroidism is about as high as that in hyperthyroidism.

7. The average nonfilament count in ten cases of hypothyroidism is higher than that in ten cases of hyperthyroidism.

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(End of Symposium)